Plant Physiology

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PREFACE

This text was developed for a one semester course consisting of two hours of lectures and six hours of laboratory per week. The material is, therefore, covered in a total of thirty lectures and, as a result, certain eliminations had to be made. It is for this reason that the subject matter has been confined (with few exceptions) to the higher plants, in opposition to the custom among older texts of covering all plants. Such a step is logical in view of the tremendous growth of microbiology as a separate science and the lack of information on the physiology of the "macro" lower plants. Similarly, the physiological responses of higher plants to their environment have been largely excluded; an advanced course on this subject is given by the author. Other advanced courses could logically be given on the individual parts of the elementary course—i.e., cell physiology, transfer of substances, nutrition, metabolism, and growth and development.

The attempt has been made, on the one hand, to give the known facts to date and, on the other, to leave the student with an appreciation of the many uncertainties that still exist in most phases of the subject. In some places the author has introduced his own terminology and concepts, rather than retain an obviously faulty and inadequate system. Similarly, where two or more hypotheses exist, the author thought it desirable to give his own analysis on the basis of the known facts. Because of the varied background of students who take this course, some elementary physics and chemistry is included in the book since no physiology course of any value can be given without referring to basic physical and chemical principles.

The author has taught the course for several years with the use of other texts. Much that is found in this book is, therefore, derived from such standard sources as Maximov, Meyer and Anderson, Thomas, Scarth and Lloyd, and Gortner, but the

borrowing was done critically and altered where it appeared necessary. For the point of view of the book, the author is to a considerable extent indebted to his physiology teacher and the guide of his earlier research work, the late Professor G. W. Scarth.

J. LEVITT

CONTENTS

Part I

INTRODUCTION

Chapter I

THE BASES OF PLANT PHYSIOLOGY

To some, plant physiology is of interest primarily as a source of basic information that can be applied practically in such allied fields as horticulture, agronomy, forestry, soil science, etc. They would prefer to study physiology as a physicist studies mathematics, purely as a tool to aid in attacking and understanding the science he is really interested in. But physiology has nowhere approached the exactness of mathematics and therefore cannot be taught in this way. Plant physiology is therefore not a satisfying study for one who must have pat answers to all questions. Any attempt to write a text that proposes to give categorical answers to most physiological problems will surely, though unintentionally, lead to a falsification of the facts and to a complete denial of the very spirit of the science.

For those with exploratory and analytical minds, plant physiology has much more than bare facts to offer. It can help them discover what are the problems of life that physiology attempts to solve; what answers to fundamental questions are plausible, and what facts are already discovered, on the basis of which we can attempt to arrive at a reasonable hypothesis; what experiments are currently being performed and what experiments should be performed. To these it will give the feeling of privileged observers watching explorations into unknown fields and learning the techniques and reasoning that are being used to attack the unsolved problems. It should encourage them to examine the known facts critically; to compare them carefully with any conclusions reached, and if reason so dictates, to reject these conclusions; and if sufficiently inspired, to launch attacks of their own in an attempt to add another grain of truth to the, as yet, small pile.

In all fields of science both theory and experiment are im-

portant. The more highly developed the field, the more attention can be paid to theory and the less to experiment, since a sound theory is able to predict experimental results correctly. In the physical sciences, theory has developed to the point where many natural laws have been established, e.g., Newton's laws of motion, the gas laws, etc. In the biological sciences, theory is still in its early stages, and few natural laws have been established.

In this respect, physiology has some advantages over other biological sciences, since its main purpose is to explain all biological phenomena in terms of physics and chemistry. Thus it inherits the laws of these two physical sciences without having to perform a single experiment. But this does not by any means free it of the necessity of turning to experimental evidence. On the contrary, in order to determine which of these laws control a physiological process, it is usually necessary to perform many carefully planned experiments. Since in most phases of physiology experimental data have not yet succeeded in conclusively establishing a theory, it is necessary to consider both the theory and the various kinds of evidence on which it rests. It is not, then, scientifically permissible to teach even an elementary physiology course in the same cut-and-dried fashion that a course in the physical sciences can be taught. This, however, has the advantage of exposing the student not only to the precepts of science but to its methods as well. Consequently, a well-taught course in physiology may quite conceivably give a better insight into science than what are usually considered well-taught courses in physics and chemistry.

In all fields of physiology, then, there are two main sources of information: (1) physical and chemical laws and (2) direct experimental evidence. Only when these two sources of information lead to the same conclusion can we be fully confident of our conclusions. In many cases, however, physical or chemical laws by themselves clearly indicate which of several conclusions are plausible and which are impossible. Two such laws that are particularly useful to the physiologist are the first and second laws of thermodynamics. According to the first law, energy cannot be destroyed or created, but is simply converted

from one form to another. It is, of course, now known that energy can also be converted into mass and vice versa, but this fact has no bearing on physiological phenomena, since the amounts of energy involved would yield immeasurably small increases if converted into mass; and, of course, no conversion of mass to energy can occur at physiological temperatures (in the case of the stable isotopes normally found in plants). This means that energy-consuming processes such as growth must be fed by energy-releasing processes such as respiration. The quantities released by the latter must be at least equal to the quantities consumed by the former.

The second law of thermodynamics is particularly applicable to physiology, since it deals with the kind of energy that is available to do work, so-called free or available energy. According to this law, the heat energy of one system allows it to do useful work on a second system only if the first is at a higher temperature than the second. And this law can be applied to all other kinds of energy. There are many cases in the following chapters where this law is made use of. It will be seen that no substances can move into a cell of themselves unless they are present in higher activities (i.e., concentrations) in the medium than in the cell. Thus the laws of diffusion follow naturally from the second law of thermodynamics. It is only when the cell expends free energy that it can absorb a nutrient from a medium of lower concentration (with respect to that nutrient) than its own; that is, the cell must then "pump" the nutrient into itself. According to a corollary of the second law of thermodynamics, free energy tends toward a minimum. This explains the fact that many substances tend to concentrate at surfaces (a phenomenon known as adsorption), for in so doing they reduce the free surface energy. Thus adsorption follows from the second law.

Other physical and chemical laws can be similarly applied in order to aid our understanding of physiological processes. But care must be taken in applying physicochemical principles to plants. Anyone with the merest rudiments of physical knowledge is certain to explain frost injury of plants as due to cell rupture, for everyone knows that water expands on freezing.

Yet experiment has shown that not only does no cell rupture occur on freezing, but the cell actually contracts because of ice formation in the intercellular (air-filled) spaces at the expense of water drawn out of the cells. This does not mean that the plant changes the physical properties of water. It means that other properties of water, besides its expansion on freezing, must be considered in order to explain the behavior of plants on freezing. Which ones must be applied can be discovered only by experiment. By this method physiologists succeeded in discovering that the plant actually does "pump" substances into itself instead of waiting for them to diffuse in. Unfortunately, in spite of the yeoman service of physiologists, much experimental work remains to be done before we can hope to understand most physiological processes. Consequently, most of them can be only somewhat superficially discussed in the light of the incomplete evidence that is available, and it must always be remembered that the explanations are only working hypotheses whose main purposes are (1) to describe the known facts and (2) to predict the plant's behavior. These predictions point to the direction in which further experimental work must be pushed.

Chapter 2

THE LIVING CELL

The cell is both the functional and structural unit of the plant, just as the molecule is the basic unit of a chemical substance. Even the nonliving cells usually have definite physiological functions. For instance, the living cells obtain a good share of their raw materials (minerals, water, etc.) by transport through such dead cells (vessels and tracheids). But since the life processes take place in living cells, it is primarily these that must be studied to understand the physiology of the plant.

How, then, can we distinguish between living and nonliving cells? Sometimes it is necessary only to observe *streaming movement* in the cytoplasm to know that a cell is alive. This movement in one direction should be clearly distinguished from *Brownian movement*, which is a haphazard oscillation about a given point and occurs just as readily in nonliving as in living systems. Another method is by *vital staining* (Fig. 1),

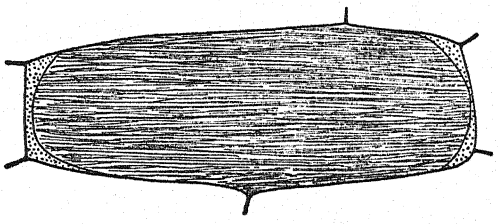
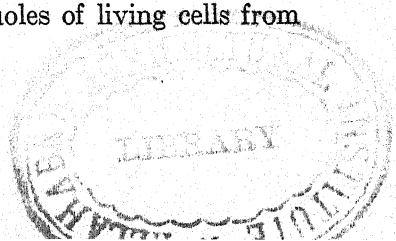


Fig. 1. Onion epidermis cell "vitally stained" with neutral red. The dark region occupying most of the cell is the stained vacuole (also called the *cell sap*). The unstained, granular, terminal caps are the cytoplasm. The lateral cytoplasm is so thin a layer as to be all but invisible. Only the cytoplasm and nucleus (not seen in the diagram) are alive.

that is, staining that involves the accumulation of dye by living but not by dead cells. Many basic dyes (salts of a dye base and an inorganic acid), such as neutral red and methylene blue, can be accumulated in the vacuoles of living cells from



weak solutions (e.g., 0.001 per cent). Dead cells never show this kind of staining and living cells lose it when killed. Some acidic dyes can stain living cytoplasm, though this usually leads to injury. A third characteristic of living cells is that they can be plasmolyzed. If a living cell [Fig. 2 (1)] is placed in a weak solution it may expand because of uptake of water [Fig. 2 (2)]. If transferred to a moderately strong solution, e.g., about 10 per cent sugar [Fig. 2 (3)], it shrinks and plasmolysis occurs.

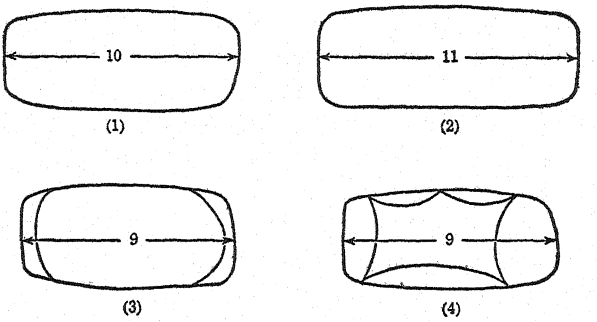


Fig. 2. Behavior of a living cell originally 10 units long in solutions of different concentrations. (1) 9% sugar (slightly hypotonic), (2) 2% sugar (hypotonic), (3) 10% sugar (hypertonic), (4) 20% sugar (hypertonic). Isotonic sugar solution between 9 and 10%. Plasmolysis in (3) and (4).

Transfer to a still stronger solution [Fig. 2 (4)] fails to cause any further shrinkage of the wall, though the plasmolyzed *protoplast* contracts still further. If transferred back to a weak solution, the protoplast expands (it deplasmolyzes) to the size it originally possessed in that solution. If the cell shrinks to its minimum size with little or no plasmolysis when placed in a solution, this solution is *isotonic* (of the same strength as the cell sap). Solutions that cause plasmolysis are *hypertonic*, those that fail to produce any trace of plasmolysis are *hypotonic*. If the cell wall is cut during strong plasmolysis and the cell is then transferred to a weak solution, the protoplast may actually swell enough to come completely out of the wall (Fig. 3).

Thus it is possible to release the inside of a cell from its surrounding wall. The empty wall shows no signs of life, but the

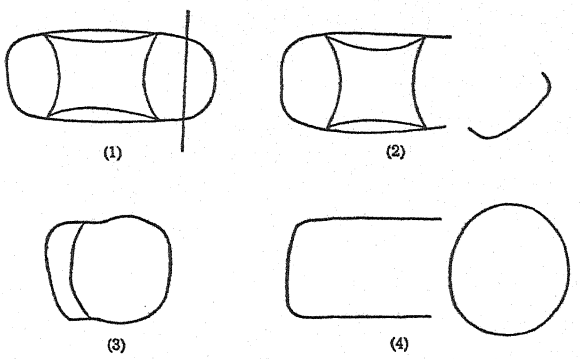


Fig. 3. Method of obtaining free protoplast. (See Levitt, Scarth, and Gibbs.) (1) Onion cell is plasmolyzed in 20% sugar then cut with a razor blade. (2) Open cell. (3) Protoplast swelling in 10% sugar. (4) Empty cell wall and spherical free protoplast.

freed protoplast will expand in weak solutions and contract in strong solutions (Fig. 4) just as in the case of the intact cell. Obviously, even the living plant cell consists of a nonliving part (the cell wall) and a living part (the protoplast). The protoplast itself consists of a protoplasmic layer surrounding a vacuole, and since some living cells exist without any vacuoles, the protoplasm is the only essential component of a living cell. Yet the nonliving cell wall and vacuole have pronounced physiological significance, since they markedly affect the protoplasm, e.g., its water content, growth, etc.

Nevertheless, the most important part of the cell to the physiologist is the living protoplasm, for here are synthesized all the multitude of organic chemical substances found in the plant. Some of the substances are also broken down to simpler ones in the protoplasm. In the former case energy is absorbed, in the latter it is released. In order for such syntheses and breakdown reactions to occur, the plant must be able to take up gases from the air, as well as water and minerals from the soil, and to give off waste gases. These processes are dealt with in a branch of physiology that may be called *transfer of substances*. The minerals required are so numerous and important that they are studied in a branch of physiology called *mineral nutrition*. The syntheses and breakdown reactions form another branch

called *metabolism*. If all these three branches of physiology are understood, we are then in a position to study the *growth and development* of the plant. But it should be realized that though such a subdivision of plant physiology into four branches is convenient for purposes of study, all the processes are interrelated and take place simultaneously in the plant.

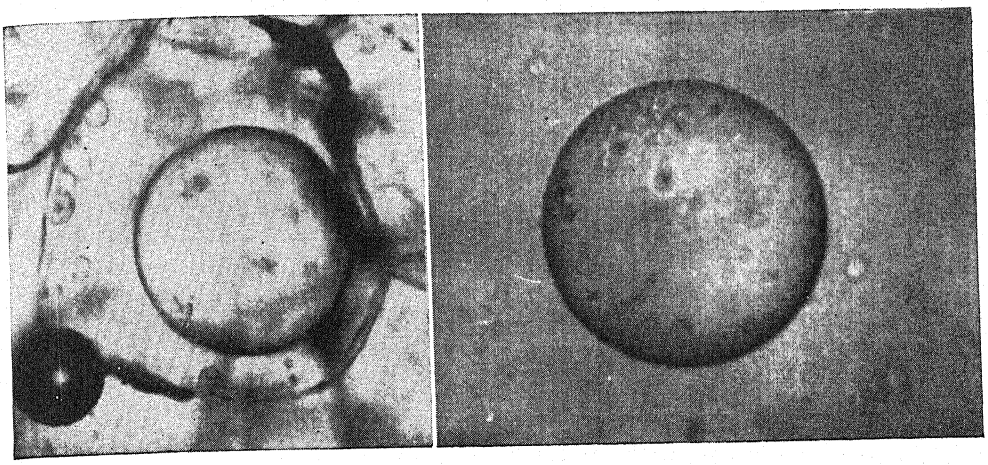
Since these branches of physiology depend, in the last analysis, on the properties of living protoplasm, they cannot be adequately dealt with until the physics and chemistry of protoplasm are understood. The gross analysis of protoplasm is possible in the case of animals and of some lower plants (e.g., slime molds) which yield masses of protoplasm without cell wall or vacuole (Table 1).

TABLE 1: GENERALIZED PROTOPLASMIC ANALYSIS
[Adapted from Sponsler and Bath]

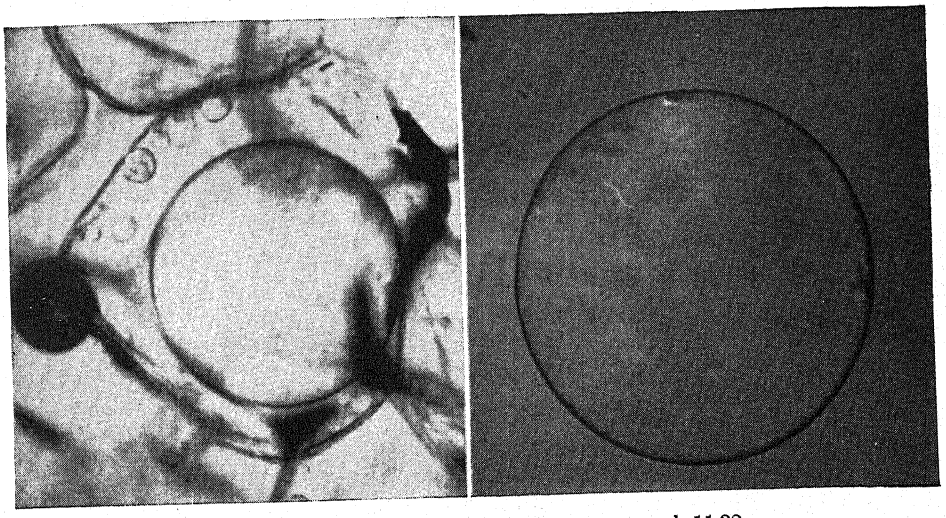
Substance	*Per cent of fresh weight*	*Approximate relative number of molecules*
Water	85–90	18,000
Proteins	7–10	1
Fatty substances	1–2	10
Other organic substances	1–1.5	20
Inorganic ions	1–1.5	100

Of course, this does not mean that it would be possible to synthesize protoplasm by mixing these substances in the above proportions. In other words, there are many essential physical and chemical properties of protoplasm not revealed by such gross analyses. If, for instance, the cell sap is mixed with the protoplasm by grinding up cells of leaves, fruit, etc., at least some of the proteins will usually coagulate. This is partly due to the high acidity of the cell sap. Yet this same cell sap is surrounded by the nonacidic protoplasm in the living cell. Therefore the protoplasm must be able in some way to maintain its own lack of acidity though in contact with the acid vacuole, as for example, by forming a barrier that keeps the vacuole contents from penetrating into it.

It is not only in acidity that the vacuole differs from the protoplasm, but in many other chemical and physical properties as well. Though the vacuole resembles the protoplasm in



(a) cell b 13.1 (b) cell b 11.1



b 13.38 b 11.32

Fig. 4. Deplasmolysis of onion protoplasts (a) inside cell wall (cell b 13); (b) free from cell wall (cell b 11). b 13.1 and b 11.1 contracted in a strong salt solution; b 13.38 and b 11.32 expanded in a weak salt solution. (See Levitt, Scarth, and Gibbs)

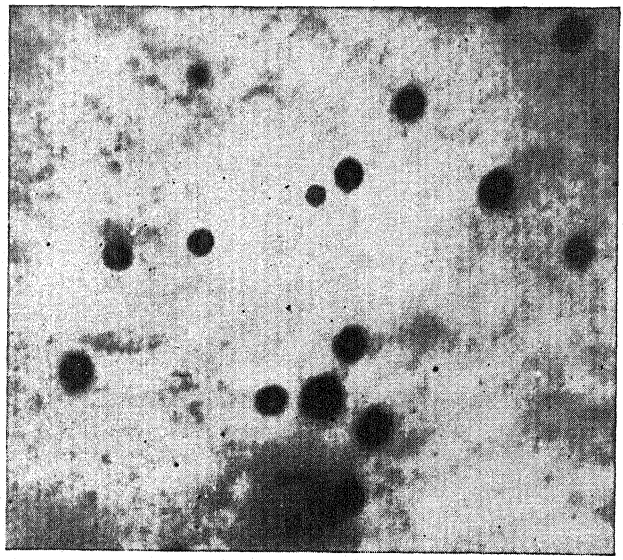


Fig. 5. Electron micrograph of potato mitochondria (× 20,000). For similar micrographs of plastids see Algera et al.

its high water content, and in consisting of a solution of organic and inorganic substances, it usually does not contain proteins and fatty substances. Salts of organic acids and sugars are probably always present in the vacuole, usually accompanied by phenolic substances such as tannins, or other organic substances such as mucilages. But the vacuole contents are much more variable than those of the protoplasm, and therefore no general analysis can be given. This is because the vacuole is the repository of (1) any substances that accumulate in excess from the outside and (2) any substances produced in excess by the protoplasm. Heavily fertilized plants may accumulate nitrates or phosphates in their vacuoles; halophytes (plants that grow in high-salt soils) store large quantities of sodium salts in their vacuoles; and as seen above vital staining with neutral red involves accumulation in the vacuole. Similarly many plants deposit excess calcium and organic acid as insoluble crystals in their vacuoles, or excess of the breakdown products of protein in the form of asparagine, or in the case of plants with specialized metabolism, alkaloids. Some of the stored substances may be later reutilized (e.g., nitrates, organic acids, asparagine, sugars); others may remain permanently in the vacuoles (e.g., alkaloids, sodium salts).

Physically, the protoplasm possesses visible structure as opposed to the optical homogeneity of the vacuole. Besides the *nucleus*, there are two smaller but microscopically visible structures (Fig. 5): the *plastids* and the still smaller (0.1–0.5μ) *mitochondria;** and another kind of structure too small to be seen with the optical microscope: the *microsomes*. A large part of the metabolism of the plant takes place in or on these structures. Carbohydrate synthesis occurs in the plastids, while breakdown of carbohydrate or its products takes place in the mitochondria. There is some evidence that protein synthesis occurs in or on the microsomes (Brachet).

The green chloroplasts can be separated from protoplasm

* The mitochondria of the biochemist are generally spherical particles, those of the histologist elongated (Guilliermond). These may or may not be the same. The ones referred to in this text are those separated from the tissue by biochemical methods. 1μ (micron) = 0.001 mm.

and still carry on part of the process of photosynthesis, as will be described later. The mitochondria can be separated and still carry on part of the process of respiration (Bonner and Millerd). But in both cases these processes can go to completion only if the other constituents of protoplasm are present. Consequently the minimum essentials for the complete life processes of the plant are unaltered protoplasm with both the microscopically visible and invisible components intact.

Among the many kinds of proteins in protoplasm are the *nucleoproteins*, which are combinations of proteins with organic compounds of phosphoric acid called nucleic acids (pentose nucleic acids, or PNA, and desoxypentosenucleic acids, or DNA). These occur in the structural components of protoplasm: nucleus, plastids, mitochondria, and microsomes. That the nucleoproteins are perhaps the most fundamental substances of life is indicated by the fact that the smallest living entity, the virus, consists solely of nucleoproteins. Similarly the units of heredity in all organisms, the genes, are also nucleoproteins.

The *phospholipids* are the main fatty substances of protoplasm. They are confined to the structural components (the plastids, mitochondria, microsomes) and the two surface layers of the protoplasm. However, the actual proportions of these substances are not the same in the different structures.

These structural components are suspended in the matrix of the cytoplasm, which is essentially made up of a protein solution or gel. This matrix is usually considerably more viscous than the vacuole. Since viscosity and gel formation are characteristic of the colloidal state, protoplasm is obviously a colloid. The nonliving parts of the cell also show colloidal properties. The vacuole, though optically homogeneous and mobile, usually possesses enough colloidal material (e.g., tannin) to accumulate dyes such as neutral red, staining more or less strongly if the cell is alive. The wall is a colloidal gel of cellulose and other large-moleculed organic substances. Though these substances are insoluble in water, they imbibe it readily in the same way that a gelatin gel does.

All three components of the living cell—wall, protoplasm,

and vacuole—contain ample water which can pass readily from one to the other. The physical chemistry of aqueous systems (e.g., diffusion, osmosis, etc.) is therefore of paramount importance in plant physiology. The lines of separation between these three components of the cell are known as interfaces. Special physical forces known as surface tension or interfacial tension operate at such interfaces. Substances tend to become concentrated at such surfaces, producing the phenomenon of adsorption.

It is now apparent that in order to understand more about the properties of protoplasm, we must consider such purely physical and chemical concepts as acidity, colloids, permeability, diffusion, osmosis, adsorption, etc. It is, in fact, the ultimate goal of physiology to describe all life processes and explain them in terms of physics and chemistry. Some aspects of physiology therefore fall naturally into the fields of biophysics or biochemistry. But the two overlap and are therefore often difficult to separate. For convenience, in this text, all those aspects of physiology that are primarily biophysical or in the borderline field of biophysical chemistry will be dealt with in Part II; all those that are mainly biochemical will be dealt with in Part III; and those aspects that involve a very intimate interrelationship of the two will be treated in Part IV.

REFERENCES

1. Algera, L., J. J. Beyer, W. van Iterson, W. K. H. Karstens, and T. H. Thung. Some data on the structure of the chloroplast obtained by electron microscopy. Biochem. et Biophys. Acta, **1**:517–526. 1947.
2. Bonner, J. and Adele Millerd. Oxidative phosphorylation by plant mitochondria. Arch. Biochem. and Biophys., **42**:135–148. 1953.
3. Brachet, J. The role of the nucleus and the cytoplasm in synthesis and morphogenesis. Symp. Soc. Exp. Biol., **6**:173–200. 1952.
4. Guilliermond, A. The cytoplasm of the plant cell. Chronica Botanica, 1941.
5. Levitt, J., G. W. Scarth, and R. D. Gibbs. Water permeability

of isolated protoplasts in relation to volume change. Protoplasma, 26:237–248. 1936.

6. SEIFRIZ, W. Protoplasm. McGraw-Hill. 1936.
7. SPONSLER, O. L. and JEAN D. BATH. Molecular structure in protoplasm. In: The structure of protoplasm, ed. G. W. Seifriz. Monograph Am. Soc. Plant Physiol. Iowa State College Press. 1942.
8. STRUGGER, S. Praktikum der Zell- und Gewebephysiologie der Pflanze. Springer. 1949.

Part II

PLANT BIOPHYSICS AND BIOPHYSICAL CHEMISTRY

Chapter 3

ACIDITY

It was stated previously that in nearly all plant cells the vacuole sap is more acid than the protoplasm. Such a conclusion can be reached only from quantitative measurements. And before quantitative measurements are possible, it is necessary to have a method of expressing acidity quantitatively.

By definition, the greater the concentration of H ions,* the more acid a solution is; the greater the concentration of OH ions, the more alkaline it is. This is usually expressed as follows:

$$\text{acidity} \propto [H^+], \qquad \text{alkalinity} \propto [OH^-]$$

where the above symbols stand for concentration in gram-atoms per liter of H and OH ions, respectively. In aqueous solutions, $[H^+] \times [OH^-] = K$. Therefore the $[H^+]$ alone reveals both the acidity and alkalinity. Since K in the above equation is 10^{-14}, a solution that is neutral (neither acid nor alkaline), and that therefore has equal concentrations of H and OH ions, must have an $[H^+] = 10^{-7}$ (1/10,000,000) gram-atoms per liter.

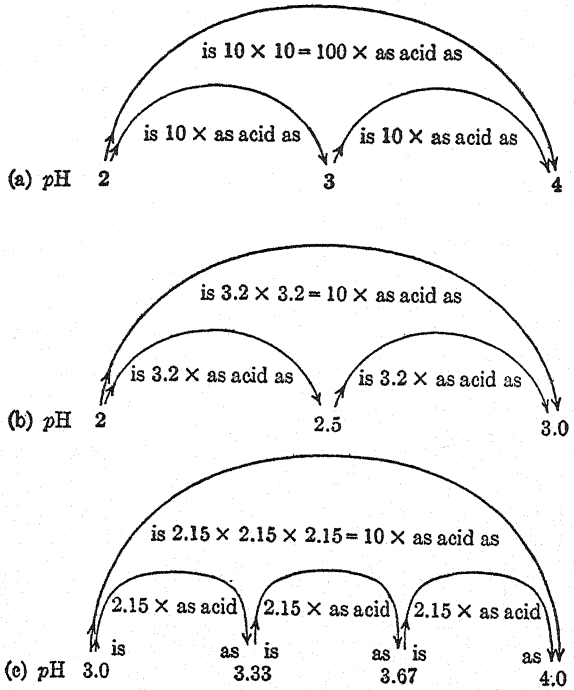
The range of acidity for the cell sap of plants is from about 10^{-1} to 10^{-7} gram-atom of H ions per liter, the one extreme being a million times as acid as the other. In order to cover such a large range conveniently, it is necessary to use an exponential method of expressing the values. Consequently, the negative logarithm of the H ion concentration is used and is called the pH. Thus pH is $-\log [H^+]$ or $\log (1/[H^+])$; conversely $[H^+] = 1/(\text{antilog } p\text{H})$. Expressed in another way, if $[H^+] = 10^{-x}$, then $p\text{H} = x$.

In terms of pH, then, the range of values for cell sap is from about 1 to 7, the former being a million times as acid as the latter. Since each whole number represents an acidity 10

* More correctly called hydronium ions (H_3O^+), since each H^+ is hydrated.

times as great as the succeeding number, the unit change in *p*H represents a smaller and smaller change in acidity as neutrality (*p*H 7) is approached. Thus a change in acidity on advancing from *p*H 1 to 2 and from *p*H 6 to 7 in each case represents a reduction in acidity to 0.1, yet the actual decrease in acidity is 0.1 − 0.01 = 0.09 gram-atom of H ions per liter in the first case, and only 0.000001 − 0.0000001 = 0.0000009 gram-atom of H ions per liter in the second case. Consequently, the former unit increase in *p*H represents 100,000 times as great a drop in acidity as the latter.

Just as a unit decrease in *p*H represents a 10 times increase in acidity, so a fraction of a unit is a definite multiple of a higher one, for example,



Acidity, as defined above, is a measure of actual H ion concentration. Besides this *true acidity*, there is the *titratable acidity*, which cannot be predicted from the H ion concentration (Table 2). Thus the normality (or titratable acidity) of

lemon juice (mainly citric acid) is 10 times that of Begonia leaf juice (mainly oxalic acid), but the latter has a greater H ion concentration and therefore a lower *p*H. The titratable acidity includes not only the actual but also the potential H ion concentration; that is, the concentration of H atoms that may change to H ions if, for instance, some of the H ions already present are deionized by neutralization or titration with OH ions. The true acidity is expressed as gram-atoms of H ions, the titratable acidity as normality of acid. In the case of a strong acid (completely or nearly completely dissociated into ions) such as HCl or H_2SO_4, the two are practically identical;

TABLE 2: TITRATABLE ACIDITY (NORMALITY) AND H ION CONCENTRATION (EXPRESSED AS THE NEGATIVE LOGARITHM OR *p*H) OF PLANT JUICES [After Thomas]

Organ from which sap was expressed	*Normality of sap*	*p*H *of sap*
Lemon fruit	0.95	2.4
Red-black fruits of blackberry	0.23	2.7
Rhubarb petioles	0.22	3.2
Unripe grapes	0.21	3.0
Oxalis leaves	0.16	2.3
Green cooking apple	0.13	3.2
Begonia rex leaves	0.11	2.2
Begonia tuberosa leaves	0.10	2.2
Ripe tomato fruit	0.063	4.4
Ripe Worcester Pearnian apple	0.045	3.9
Celery petioles	0.025	5.2
Root of white beet	0.025	5.8

but in the case of a weak acid (with only a small fraction of the molecules dissociated) the true acidity is much less than the titratable acidity. Since *p*H is related to true acidity, a strong acid has a much lower *p*H than a weak acid of the same normality. Thus, the *p*H of 1 *N* HCl is 0.10, which is close to the ideal value of 0.00. The *p*H of 1 *N* acetic acid is 2.37 because only 0.42 per cent of the acid molecules are dissociated. This means that the 1 *N* HCl is nearly 200 times as acid as the 1 *N* acetic acid, though the same amount of alkali would be required to neutralize equal volumes of the two acids. The strength of an acid therefore depends on how much of it is ionized, and this is expressed quantitatively by the ionization constant (Table 3).

TABLE 3: DISSOCIATION OR IONIZATION CONSTANTS $K = \frac{[H^+] \times [A^-]}{[HA]}$ OF SOME ACIDS FOUND IN PLANTS AND THEIR RESPECTIVE pK
(the pH at the middle of their buffering zones)
[After Pauling and Thomas]

		K	pK
Inorganic acids			
phosphoric	1st H to ionize	7.5×10^{-3}	2.1
	2nd H	6.2×10^{-8}	7.2
	3rd H	1×10^{-12}	12.0
carbonic	1st H	0.45×10^{-6}	6.3
Organic acids			
oxalic	1st H	5.9×10^{-2}	1.2
	2nd H	6.4×10^{-5}	4.2
malic	1st H	4×10^{-4}	3.4
tartaric	1st H	9.7×10^{-4}	3.0
citric	1st H	8.7×10^{-4}	3.1
acetic		1.8×10^{-5}	4.7

Since the weak acid has nearly all its titratable H atoms in the form of potential H ions, when mixed with its salt it maintains a nearly constant pH, even though large amounts of H or OH ions are added to it. If H ions are added, they will nearly all combine with the excess anions (supplied by the salt) to form undissociated acid. If OH ions are added, they combine with the H ions from the weak acid to form undissociated water molecules, but the neutralized H ions are immediately replaced by ionization of some of the large store of potential H ions. Such a mixture of weak acid plus its salt (or weak base plus its salt) is called a *buffered* solution because of its ability to prevent changes in pH. A good example is a mixture of equimolar quantities of acetic acid and sodium acetate. A drop of concentrated HCl added to a liter of such a buffered solution has a negligible effect on the pH. A drop of concentrated HCl added to a liter of unbuffered water decreases its pH about 3.7 units (i.e., increases its acidity about 5000 times).

The buffer range of an acid can be observed from its titration curve, for it is that portion of the curve showing the most gradual rise in pH (Fig. 6). A strong acid (0.1 N HCl) also shows a region of gradual rise with only a unit increase in pH though enough alkali is added to neutralize 90 per cent of it. But this is not a true buffering action, since the $[H^+]$ is reduced

by about 0.09 gram-atom. The buffer zone of the weak acid is much higher on the *p*H scale; therefore, though a similar rise in *p*H occurs, it represents only a fraction of 1 per cent as much of a decrease in $[H^+]$ for the same addition of OH^- as in the case of the HCl. This is due to the large store of potential H ions that ionize as more alkali is added.

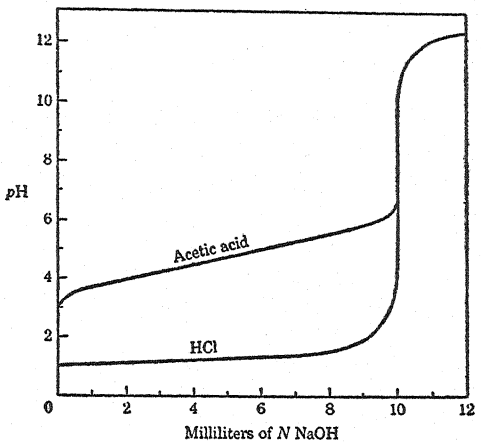


Fig. 6. Titration curves of 100 ml. portions of 0.1 *N* HCl and 0.1 *N* acetic acid with *N* NaOH. (After Michaelis)

Each buffer mixture is effective only within a certain range of *p*H. The maximum buffering of any mixture is obtained when half the acid (or base) molecules are dissociated; that is, when equal quantities of undissociated acid (or base) and H (or OH) ions are present. This occurs at a $[H^+]$ that is numerically equal to the dissociation constant K of the acid; for if an acid HA dissociates into H^+ and A^-, then $K = \frac{[H^+] \times [A^-]}{[HA]}$; therefore, when the acid is half dissociated, $[A^-] = [HA]$ and $K = [H^+]$. Since the dissociation constants of most acids are known, the optimum buffer zone for each can be easily calculated; for the mid-point of this zone will occur at the *p*H equal to $-\log K$ (the so-called pK).

In plant juices, several organic and inorganic salts and acids (Table 3) are responsible for the buffering action (Small).

Around the neutral point, phosphates and bicarbonates are the commonest buffers; on the acid side organic salts and acids such as malates (apples, peas, asparagus), citrates (tomato, citrus fruits), oxalates (tomato, plum, strawberry), tartrates (grapes), etc. Usually more than one buffer is present, e.g., malate and acetate in onion, malate and oxalate in lettuce. Most of the above substances are in the vacuole. The substances responsible for buffering of plant protoplasm are not

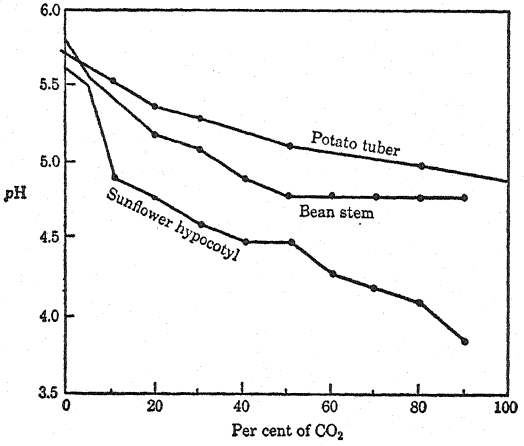


Fig. 7. Effect of CO_2 on *p*H of plant juices. (After Small)

known, though the proteins themselves have some effect. Some plant saps are not well buffered and their *p*H may be markedly shifted by addition of CO_2 (Fig. 7). In some cases, excess CO_2 may produce indirect effects leading to a rise in *p*H.

There are two main methods of measuring *p*H; one by use of *indicators* (dyes that change color at more or less specific *p*H), the other by *p*H *meters*. Each has its own virtues, though the modern, line-operated *p*H meters with glass electrodes are unequaled for accuracy, speed, and simplicity. The indicator method is still sometimes used, for instance in field work. It is accurate enough for most biological purposes. For determination of *p*H of individual cells it is the only method available, since electrodes cannot be made small enough to insert into most living cells. Naturally occurring indicators inside the cell

may sometimes be made use of; flavones turn from colorless to yellow above *p*H 8; anthocyanins are usually pink or red below *p*H 5, blue or purple above it.

By the use of these methods, the *p*H's of many plant juices have been determined. Fruit juices usually range from *p*H 2.5 to 4.5 (Table 2) though there are some exceptions (e.g., lime, *p*H 1.7). Juices expressed from other plant parts also show wide variations though they commonly fall within the range of *p*H 5.0 to 6.5. Phloem exudate has the exceptionally high value of 7–8. In plants with acid metabolism (e.g., succulents) the *p*H may be around 3.0. Even in the case of a single plant it may vary markedly. In many plants it drops at night and rises during the day. Extreme examples of such diurnal *p*H changes are found among succulents: in *Opuntia phaeacantha* (a cactus) from 5.5 at 4:00 p.m., to 1.4 at 6:45 a.m. Where the change in *p*H is small, it may be at least partly due to increases in CO_2 content at night (from respiration), and decreases during the day (due to use in photosynthesis). But such extreme changes

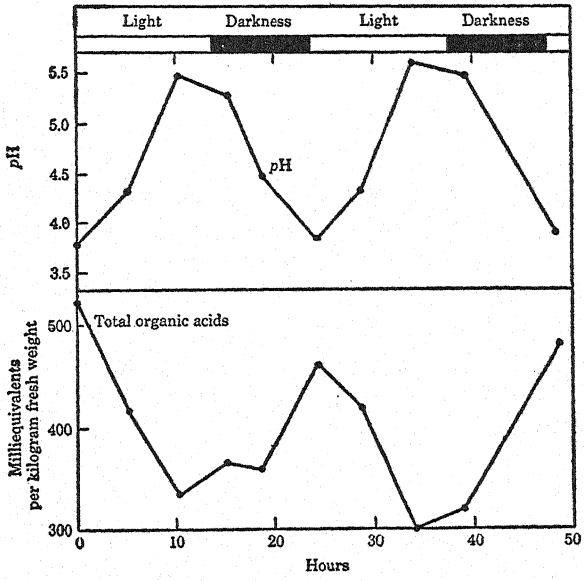


Fig. 8. Effect of light and darkness on *p*H and organic acid content of Bryophyllum leaves. (After Vickery)

as in the Opuntia and other succulents result from accumulation of organic acids in the dark and their disappearance in the light (Fig. 8).

The *p*H of plant protoplasm is much more difficult to determine, partly because of the far greater quantity of acid vacuolar sap adjacent to it. It is perhaps because of this and the *acid of injury* that often arises when protoplasm is injured, that values as low as *p*H 5.6 have been recorded for plant protoplasm. Because of these sources of error and because animal protoplasm has usually been found to be around the neutral point, it is highly probable that plant protoplasm has a *p*H around neutrality. Indirect evidence of this is the fact that when protoplasmic proteins are isolated from plants, some of them fail to remain in solution unless the *p*H is maintained above 6 to 6.5.

Judging from the range of *p*H found in the vacuole (1–7), the plant is apparently much more tolerant of acidity than of alkalinity. Even the tolerance of externally applied *p*H is not so great in the alkaline range. The most acidiphilous plants grow in soil *p*H down to 4.0 or even 3.5 in extreme cases, the alkaliphilous in soil *p*H up to 8.0 or 8.4. Thus the tolerance of alkalinity is greater than appears from vacuole measurements, but is still much less than the tolerance of acidity. This is at least partly associated with indirect effects of *p*H—e.g., the unavailability of some soil nutrients at high *p*H—rather than a direct damage of protoplasm by OH ions. According to Olson, direct damage by OH ions does not occur until *p*H 10.5, exactly the same distance from the neutral point as the *p*H (3.5) causing direct damage due to H ions.

The role of *p*H in many physiological processes will be dealt with in later sections (e.g., protein hydration, stomatal movement, enzyme action, etc.).

REFERENCES

1. Clark, W. M. The determination of hydrogen ions. Williams and Wilkins. 1928.
2. Michaelis, L. Hydrogen ion concentration. Williams and Wilkins. 1926.

3. OLSON, CARSTEN. The significance of concentration for the rate of ion absorption by higher plants in water culture IV. The influence of hydrogen ion concentration. Physiol. Plant., **6**:848–858. 1953.

4. PAULING, L. General chemistry. Freeman. 1947.

5. SMALL, J. *p*H and plants. Bailliere, Tindall & Cox, Ltd. 1946.

6. THOMAS, M. Vegetable acids in higher plants. Endeavour, *10*(**39**): 160–165. 1951.

7. VICKERY, H. B. The behavior of isocitric acid in excised leaves of *Bryophyllum calycinum* during culture in alternating light and darkness. Plant Physiol., **27**:1–17. 1952.

8. WILLARD, H. H., L. L. MERRITT, and J. A. DEAN. Instrumental methods of analysis. Van Nostrand. 1951:179–205.

Chapter 4

SPECIFIC SURFACE AND ADSORPTION

If we compare a cabbage seedling with an onion bulb, it is at once apparent that the former has more total surface, and to an even greater degree more *specific surface* (surface per unit volume). Thus, if the seedling has 12 leaves, each of which is about 9×8 cm., the area of one leaf surface is about $\frac{3}{4}(9 \times 8) = 56$ cm.^{2} If the above-ground portion weighs 25 g., the specific surface is about 50 cm.^{2} per cm.^{3} (or 50 cm.$^{-1}$), since 1 g. of fresh tissue has a volume of about 1 cm.^{3}

If the onion bulb has a diameter of 6 cm. its specific surface is

$$\frac{\text{surface}}{\text{volume}} = \frac{4\pi r^2}{4\pi r^3} = \frac{3}{r}$$

$$= 1 \text{ cm.}^2/\text{cm.}^3 \ (= 1 \text{ cm.}^{-1})$$

The above-ground portion of the cabbage seedling obviously has 50 times as much specific surface as the onion bulb.

This difference in specific surface will markedly affect all phases of the physiology of the two plants. If this were the only difference between the two, the cabbage would be able to absorb O_2 and to give off CO_2 and water at 50 times as great a rate per unit volume as would the onion bulb. Specific surface is therefore an important factor in the exchange of substances between a plant and its environment. As a result of the more rapid exchange of O_2 and CO_2, the cabbage would be able to break down and synthesize chemical substances more rapidly than the onion bulb. Because of this greater metabolic rate more energy would be available. As a result the cabbage might be expected to grow and develop more rapidly. Similarly, the temperature of the cabbage plant would become adapted to its environmental temperature more rapidly should the latter change.

This kind of reasoning is a gross oversimplification, since

there will always be many other factors that may counteract the effect of surface. Nevertheless, it cannot be denied that the specific surface of a plant may affect all phases of physiology. In line with the above reasoning, for instance, is the fact that actively growing plants and plant parts do, in general, have more specific surface than resting or dormant plants or plant parts (seeds, tubers, bulbs, etc.). Thus deciduous trees reduce their surfaces tremendously when they become dormant. In the case of evergreen trees this is not true, but that may partly account for their greater successes in climates having low enough temperatures during their dormant period to reduce their metabolism to a minimum without any reduction in surface. The large specific surface of active plants is to be expected from the laws of thermodynamics, which teach us that any increase in surface area involves an increase in free energy, i.e., energy available to do work. But even more important and much larger than the external surface of a plant is its internal surface. The above-mentioned onion bulb, for instance, consists mainly of spherical cells with an average diameter of about 0.1 mm. or 0.01 cm. This results in a much larger total protoplasmic surface than if all the protoplasm existed in one spherical mass. Since specific surface of a sphere varies as $1/r$, and since we are dealing with the same total volume, the total cell surface must be greater than the bulb surface by the ratio R/r, where R = bulb radius and r = cell radius, or 3/0.005, i.e., 600 times the bulb surface. Since the protoplasm layer has two surfaces, its total surface is 1200 times that of the bulb.

Though further calculations are unnecessary, observation of these onion cells under the microscope will reveal the existence of nuclei, plastids, and mitochondria in the cytoplasm. The total surface of all these is tremendous. Below the range of visibility are still smaller particles (microsomes) with still greater specific surface. These invisible particles are in the colloidal state.

One of the most important properties of surfaces is the tendency for substances to become concentrated there. This follows from the laws of thermodynamics. If a body is cut up into small pieces, the surface increases, and from the law of the

conservation of energy the work done in cutting the body is at least partly converted into free surface energy. This energy is commonly either in the form of electric charge or of surface tension. From the second law of thermodynamics, it follows that the free energy tends to be converted into unavailable energy. In other words, the surface charge will tend to be neutralized by opposite charges, the surface tension to be lowered by other substances. In both cases, molecules or particles that reduce surface energy will accumulate at the surface in higher concentration than in the surrounding medium. This concentration at a surface is known as *adsorption*. It is more pronounced at some surfaces than at others, and from some media than from others. In some cases there may even be *negative adsorption* if this results in a lower free energy (i.e., if the only available particles have the same kind of charge as the surface or if they raise surface tension).

Water consists of *polar* molecules, i.e., molecules with one end

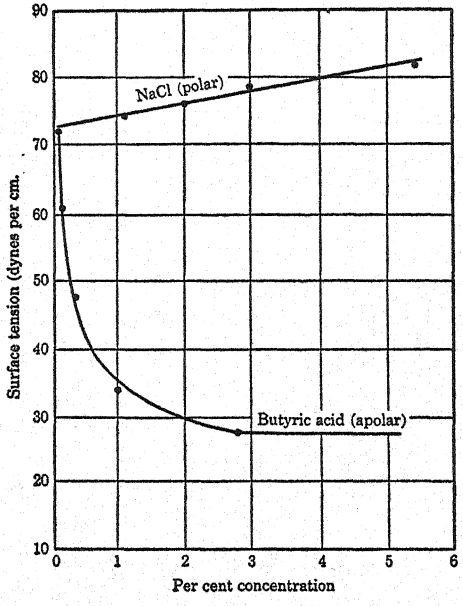


Fig. 9. Surface tension of solutions of polar (salts and sugars) and apolar (many organic) substances. (After Freundlich)

electrically negative with respect to the other. Other substances that also consist of polar molecules tend to attract water molecules electrically and can be thought of as being pulled into the body of an aqueous solution (negative adsorption). Salts (and sugars) are polar substances and therefore do not concentrate at the surface. Consequently they do not reduce the surface tension of water. They are therefore said to be surface inactive, though they may actually slightly increase surface tension (Fig. 9). Most organic substances are more or less apolar and therefore are not so strongly attracted to the water molecules as the water molecules are to each other. As a result they can be thought of as being "pushed" to the surface, where they reduce the surface tension due to the small molecular forces of attraction between them. Such substances are said to be surface active. Proteins are surface active and therefore form an adsorbed layer around latex particles in rubber-producing plants. But proteins do possess definite electrical properties and therefore are not nearly so surface active as fatty substances. Consequently, in the case of living protoplasm there is a far greater concentration of lipids than of proteins at the surface.

Adsorption has proved to be important in the case of uptake of nutrients from the surrounding medium by plant roots. There is strong evidence that the first step in the uptake is adsorption at the surface of the roots. Now it has been found that

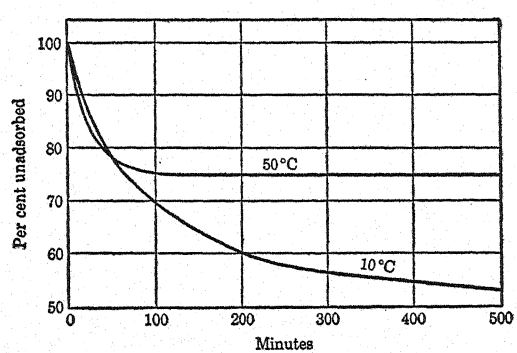


Fig. 10. Effect of temperature on rate of adsorption of Congo red by paper. (After Belehradek)

in weak solutions the uptake is more or less proportional to the external concentration, but that in more concentrated solutions it is practically independent of the external concentration. This is believed due to saturation of the surface by the adsorbed nutrient at relatively low concentrations (Robertson).

Temperature has a pronounced effect on adsorption. The amount adsorbed markedly decreases with rise in temperature. This can readily be understood, since the higher the temperature, the greater the kinetic energy of the adsorbed molecules, and the more easily they can escape from the surface. However, temperature has the opposite effect on the time required for adsorption equilibrium to be attained; this decreases with rise in temperature (Fig. 10).

REFERENCES

1. Belehradek, J. Temperature and living matter. Protoplasma Monograph 8. Gebrüder Borntraeger. 1935.
2. Freundlich, H. Colloid and capillary chemistry. Trans. by H. S. Hatfield. Dutton. 1922.
3. Gortner, R. A. Outlines of biochemistry. Wiley. 1949.
4. Robertson, R. N. Studies in the metabolism of plant cells. I. Accumulation of chlorides by plant cells and its relation to respiration. Austral. J. Ex. Biol. and Med., **19**:265–278. 1941.
5. Scarth, G. W. and F. E. Lloyd. Elementary course in general physiology. Wiley. 1930.

Chapter 5

COLLOIDS

The most obvious factor in adsorption is the extent of surface —the greater the surface per unit volume (i.e., the specific surface) the greater the amount adsorbed per unit volume. Adsorption therefore becomes more pronounced the more finely divided the adsorbent. It reaches a maximum, in fact, in the *colloidal* range where particle sizes are below visibility with the optical microscope but above ordinary molecular size (Table 4). It is for this reason that adsorption must play such an important role in the living cell and particularly in protoplasm which consists largely of substances in the colloidal state.

TABLE 4: SIZE LIMITS OF COLLOIDAL PROPERTIES
[Adapted from Scarth and Lloyd]

2μ	200 mμ	5 mμ	0.1 mμ
Visible with the ordinary microscope	Visible with the ultramicroscope and the electron microscope	Invisible even with the electron microscope	
Suspensions	Colloids	Molecular dispersions	
Filterable	Dialysable	Nondialysable except by semipermeable membranes	
Unstable—settle out on standing	Relatively stable	Stable	

1 mm. = 1000μ (microns), 1μ = 1000 mμ

Since the colloidal state depends simply on the particle size, any substance can (at least theoretically) be a colloid. There is, of course, no sharp line between colloids and noncolloids. Any particles that fall in the range of about 1–5 mμ to 0.1–0.2μ are usually considered to be colloidal. Sometimes the upper limit may be extended to 0.5μ since certain systems with particles

of this size are stable. The lower limit includes some molecules, since these may be large enough to take on colloidal properties. This is particularly true of proteins.

Colloidal systems really consist of two components, the *disperse phase* (particles) and the *dispersion medium*. Since both of these may be in any of the three states of matter (solid, liquid, and gas), there are nine possible combinations. Of these only one cannot represent a colloid, viz., gas disperse phase in gaseous medium, since all gases are molecularly miscible in all proportions. Of the eight remaining possible combinations, only one, the solid disperse phase in a liquid medium, is important in physiology. And the only liquid medium of physiologic significance is water. Consequently the following discussion applies primarily to aqueous colloidal dispersions.

Properties of colloids

There are many ways in which colloidal dispersions differ from molecular dispersions or *true solutions*. All these differences depend on the particle size. Thus the colloidal particles are large enough to scatter light. As a result, colloidal dispersions frequently show a *Tyndall effect;* if a beam of light is shone on the dispersion, the path of the beam can be seen from either side at right angles to the direction of the beam. But such light scattering is not possible when the colloidal particles have a refractive index that does not differ markedly from that of the dispersion medium, that is, when the particles are highly hydrated as in the case of many proteins.

Another result of their relatively large particle size is the slow rate of diffusion of colloids, as little as 0.001 the rate of small molecules (Table 5).

TABLE 5: RELATIVE DIFFUSION RATES (OR DIFFUSION COEFFICIENTS) OF MOLECULARLY AND COLLOIDALLY DISPERSED SUBSTANCES
[After Gortner]

Dispersion	*Substance*	*Diffusion coefficient*
molecular	nitric acid	2.1
	sucrose	0.31
colloidal	nuclear gold (1.7 mμ)	0.27
	egg albumin	0.059
	antitetanus serum	0.0021

The osmotic effects of colloidal solutions are small, again because of the large particle size (Table 6).

TABLE 6: OSMOTIC PRESSURES IN ATMOSPHERES OF A MOLECULARLY DISPERSED (DEXTROSE) AND A COLLOIDALLY DISPERSED (SERUM ALBUMIN) SUBSTANCE

Per cent solution	*Dextrose*	*Bovine serum albumin*
5	6.7	0.013
10	13.5	0.032
20	27.0	0.12

The colloidal particles are too large to pass through the pores of many membranes that are easily penetrated by molecularly dispersed salts and sugars. This fact is made use of to free colloidal systems of crystalloids. When confined in such a membrane which is suspended in water, the crystalloids diffuse out, leaving the colloids behind. This process is known as *dialysis*. A similar process including the application of pressure is known as *ultrafiltration*.

Colloids may differ from each other in other ways besides size. Some may be nonspherical, and this may lead to other properties such as gel formation. In spite of their large particle size, some may be electrolytes and due to their dissociation become charged. Proteins supply examples of both of these properties.

Stability of colloidal dispersions

Molecular dispersions are stable because of the high kinetic energy of the molecules. When this energy is reduced, that is, when the molecules crystallize, they precipitate. In the same way, colloidal particles may aggregate to form larger particles, which will then precipitate. If the colloid does not precipitate, it is stable, and since the particles do not possess the high kinetic energy of molecules, the colloid must owe its stability to some other property that prevents aggregation. There are two main factors that contribute to its stability, *charge* and *hydration*.

The charge of a colloidal particle is due either to the capture of an ion or to ionization (dissociation) of the colloid. Colloidal

bases (e.g., alkaloids, basic dyes, and hydroxides of metals) become positively charged in water. All other colloids are negative in water. Proteins are amphoteric (either positively or negatively charged) depending on whether they dissociate as bases or as acids. At some point between, the *isoelectric point*, their net charge is zero, and they are therefore least stable. Some proteins (the albumins) are *isostable;* that is, their own ionization supplies a sufficient net charge. Others (globulins) need other ions and therefore are stable only in salt solutions. An unstable colloid may therefore be dispersed by addition of sufficient ions (i.e., by salts). This is called *peptization.* But if too high a salt concentration is used the colloid may be discharged and precipitated. This is called *flocculation.* The higher the valence of the oppositely charged ion, the smaller the concentration of salt required to flocculate a colloid (Table 7).

TABLE 7: CONCENTRATION OF SALTS REQUIRED TO FLOCCULATE (PRECIPITATE) SOLS [Adapted from Scarth and Lloyd]

a) *Negative Sol*		b) *Positive Sol*	
Salt	*Millimols*	*Salt*	*Millimols*
KCl	50	KCl	80
$BaCl_2$	0.70	K_2SO_4	0.28
$AlCl_3$	0.09	$K_4Fe(CN)_6$	0.08

The second cause of stability is hydration. Aqueous colloids may be roughly classified into *hydrophilic* and *hydrophobic* colloids, though there is some gradation between the two groups. The hydrophobic are less stable and can be flocculated by low concentrations of electrolytes (Table 7). The hydrophilic cannot be flocculated, but may be *salted out* by high salt concentrations. The effectiveness of salts varies with the different ions in a very definte order which is known as the *lyotropic* (or Hofmeister) series (Table 8).

TABLE 8: ORDER OF EFFECTIVENESS OF IONS IN SALTING OUT HYDROPHILIC SOLS; LYOTROPIC SERIES [After Scarth and Lloyd]

Cations: $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$
Anions: $SO_4^= > F^- > Cl^- > NO_3^- > Br > I^- > CNS^-$

Proteins are typically hydrophilic colloids. They cannot be flocculated by low concentrations of salts but can be precipi-

tated by high concentrations, e.g., by saturation or half saturation with $(NH_4)_2SO_4$. Some hydrophilic colloids form aqueous sols (i.e., have the physical properties of a liquid), others form more or less rigid gels. Protoplasm itself is intermediate and exhibits the properties of both sols and gels; it shows streaming and brownian movement as would be expected in a sol, but both of these movements may be stopped by removal of some of its water, converting it into a rigid gel.

Properties of gels

Since protoplasm has the properties of gels, it is important to know what these are. In spite of the more or less rigid nature of aqueous gels, their water content is usually high, and this insures just about as rapid diffusion of water-soluble substances through them as through water itself. The following are their main properties:

1. *Swelling or imbibition pressure.* Most aqueous gels are

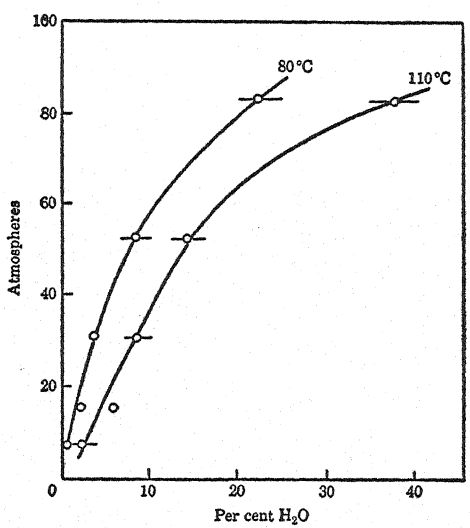


Fig. 11. Per cent bound water held by the mold *Aspergillus niger* after growth in media containing 0 to 50% sugar, and therefore having osmotic potentials (see Chap. 6) of nearly zero to 100 atm. The bound water is that held at room temperature in an evacuated desiccator with a relative humidity of practically zero. Much of this bound water can be driven off at 80°C. (upper curve), more at 110°C. (lower curve).

highly hydrophilic. Due to the large adsorptive forces, they may have imbibition pressures as high as 1000 atm.; that is, they may absorb water against water-removing forces of this value; or they may hold some water against pressures of 1000 atmospheres in a hydraulic press. This is true also of seeds, due to their gel nature. Proteins may retain as much as 10 g. water per 100 g. protein at 100°C. Water that is adsorbed strongly by colloids is sometimes called *bound water*. The amount of such "bound water" held by tissues may vary with the conditions of growth (Fig. 11), and the water is retained against forces of evaporation of well over 1000 atm.

2. *Hysteresis.* If several gelatin gels are made up of different concentrations and dried, when allowed to reimbibe water each will tend to regain its original concentration (Fig. 12). This is due to the structure of the gel. The colloidal particles are needle-shaped and cross each other at definite points, forming a "brush-heap" type of structure. The water is held in the interstices between the *micelles* (particles). Since these micelles tend to maintain a constant position with reference to each

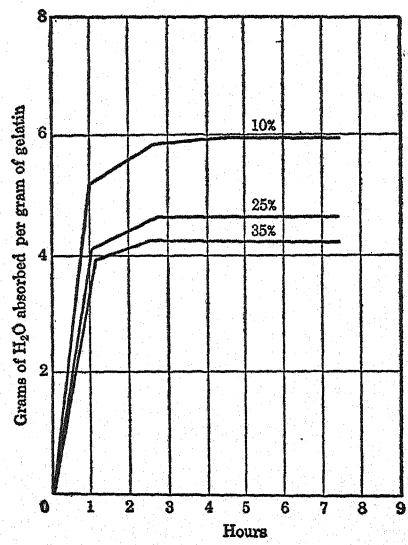


Fig. 12. Imbibition curves of gelatin made up of 10, 25, and 35% gels, dried to 3% moisture, then allowed to reimbibe water. (After Gortner)

other, that is, they act as though fastened together at the points of contact, the dilute gels will have larger interstices for holding water than will the concentrated gels. This phenomenon may be of fundamental importance in the case of protoplasm. If a plant has grown under low moisture conditions, its protoplasm may conceivably have this tight kind of structure and perhaps be unable to imbibe as much water as if it had grown under conditions of high moisture. This may possibly be one reason why such plants are sometimes permanently set back by early droughts, even though not obviously injured.

3. *Syneresis*. Sometimes the ability of a gel to hold water decreases with age, and free water (or solution) is then liberated. This is called syneresis. Syneresis may also conceivably happen in the case of senescent cells. The vacuole would then enlarge at the expense of the protoplasm.

4. *Thixotropy*. Some gels may act as sols if stirred or shaken. After this treatment they may pour quite readily. On standing they become gels again and will not pour. This reversible sol $\rightleftharpoons$ gel transformation is called thixotropy. By use of microneedles, it is possible to show that protoplasm is thixotropic.

REFERENCES

1. Gortner, R. A. Outlines of biochemistry. Wiley. 1938.
2. Scarth, G. W. and F. E. Lloyd. Elementary course in general physiology. Wiley. 1930.
3. Todd, Glenn W., and J. Levitt. Bound water in Aspergillus niger. Plant Physiol., **26**:331–336. 1951.

Chapter 6

DIFFUSION AND OSMOSIS

In the living and growing plant, there is a continuous transfer of materials from cell to cell, from tissue to tissue, from organ to organ, from the plant to and from its environment. In purely physical systems, transfers of both solvent and solute occur as a result of molecular movement, i.e., by the process of *diffusion*. Consequently, diffusion must be considered as a possible factor in the movement of both water and solutes in the plant. Since cells possess semipermeable membranes, the movement of water must involve *osmosis* (diffusion through semipermeable membranes).

Diffusion

Since diffusion is due to the movement of the individual molecules, any factor controlling the rate of diffusion does so by altering the total free energy of the substance, because of a difference (a) in the speed of the molecules (1 and 2 below) or (b) in the number per unit volume (3 and 4). The rate of diffusion of a solute therefore depends on:

1. *The size of the diffusing particles.* For gases and small molecules v is proportional to $1/m$, where v is velocity and m is mass. For large molecules and colloidal particles the relation is more nearly v is proportional to $1/r$, where r is the radius of the particle.

2. *Temperature.* The rate of diffusion increases with the temperature; for example, NaCl diffuses in water 4 times as rapidly at 50°C as at 0°C.

3. *The concentration gradient.* Diffusion occurs from a high to a low concentration, the greater the difference (per unit distance) the more rapid the diffusion.

4. *Permeability of the medium.* The dissolving power of the medium controls the number of particles that can move abreast

in it. Therefore diffusion is proportional to solubility (or permeability), when the solute is present in excess of saturation.

For a particular solute diffusing through a particular medium at a constant temperature, the specific diffusion rate D is the number of moles that diffuse across unit area in unit time under a concentration gradient of unity. If two different concentrations of a solution exist on opposite sides of a permeable membrane (Fig. 13) the rate is given by Fick's law of diffusion:

$$\frac{s}{t} = Da\frac{C_1 - C_2}{x}$$

or, expressed as a differential equation,

$$\frac{ds}{dt} = Da\frac{dc}{dx}$$

where s = amount of substance diffusing (moles)
t = time (sec.)
D = coefficient of diffusion or specific diffusion rate
a = area of membrane (cm.^{2})
C_1 = higher concentration (moles per liter)
C_2 = lower concentration
x = membrane thickness (cm.)

$\frac{C_1 - C_2}{x}$ or $\frac{dc}{dx}$ = concentration gradient

Here D is a constant for any one diffusing substance and medium if the temperature is constant, i.e., if three of the factors mentioned above are constant. If more than one sub-

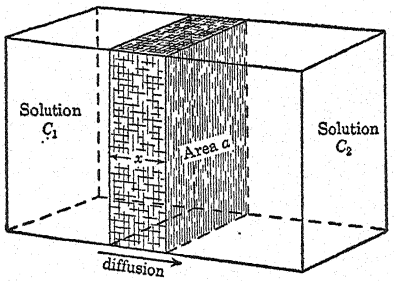


Fig. 13. Diffusion of a substance from a region of higher to one of lower concentration. (After Scarth and Lloyd)

stance is present, each moves according to its own concentration gradient and coefficient of diffusion.

Since so many factors are involved in the diffusion of a substance, it is sometimes more convenient to express all these in one general term, the activity or free energy of the molecules. This method is particularly useful in the case of a solvent whose "concentration" is usually so near to 100 per cent as to be difficult of accurate measurement. Thus the solvent will always move from a region of higher activity or free energy to one of lower activity or free energy.

Osmosis and osmotic pressure

When a substance (the solute) is added to a liquid (the solvent) in which it is soluble, the free energy of the solvent molecules is decreased. This decrease in free energy (or activity) of the solvent molecules lowers its vapor pressure and freezing point, and raises its boiling point. It also gives rise to the phenomena of osmosis and osmotic pressure (the hydrostatic pressure due to osmosis). Thus if the solution is separated from pure solvent by a membrane permeable to solvent, but not to solute (a semipermeable membrane), the solvent molecules will diffuse from the region where their free energy is higher (the pure solvent) to the region where their free energy is lower (the solution). This selective diffusion of solvent is called *osmosis*, and it gives rise to *osmotic pressure*, which is determined by the pressure necessary to stop osmosis. Osmosis will continue until the free energy difference disappears, as a result of either concentration changes or pressure changes (Fig. 14). Whereas a rise in concentration of solute decreases the activity of the solvent molecules, a rise in pressure increases it. In case (1) of Fig. 14, both concentration and pressure changes would occur; that is, water would enter the solution, causing its level to rise above that of the solvent. This would produce a hydrostatic pressure and at the same time it would dilute the solution. Both changes would increase the free energy of the water molecules in the solution, until at equilibrium this would reach the value for water molecules in the pure solvent.

In case (2), the free energy of the water molecules in the

sugar solution is raised by application of an external pressure p. If this pressure is increased until there is no diffusion of water in either direction (i.e., the water molecules move equally rapidly in both directions), then p must be equal to the osmotic

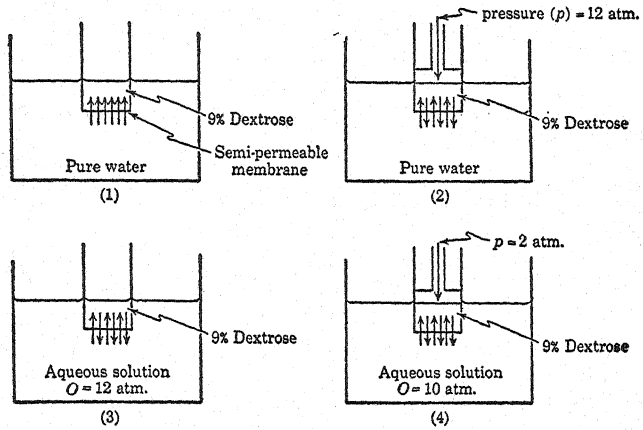


Fig. 14. Osmosis and osmotic pressure in an artificial cell containing 9% dextrose (corn sugar) separated from water or solution by a semipermeable membrane.

pressure produced by the system of 9 per cent dextrose (corn sugar) separated from pure water by a semipermeable membrane. In this case, p will have to be 12 atmospheres. Consequently, a 9 per cent dextrose solution (i.e., 0.5 M or half molar dextrose) is capable of producing an osmotic pressure of 12 atm. This then is the *osmotic potential* (O) of 0.5 M dextrose. It is transferred into an actual pressure only under specific conditions as in case (2) above. It should be realized that the osmotic potential of a solution and the activity of the solvent are inversely related; that is, water will move from a region of low osmotic potential to one of high osmotic potential. The pressure p is transmitted hydrostatically to the walls of the container, and according to Newton's third law of motion, an equal and opposite pressure must be exerted by the walls of the container on the sugar solution. This is called the *wall pressure.*

In case (3), the 9 per cent dextrose solution is separated by the semipermeable membrane from a solution of another sub-

stance with an osmotic potential of 12 atm. As in case (2), equilibrium again exists without the application of any external pressure because both solutions have the same osmotic potential.

In case (4), the two osmotic potentials are not equal, yet equilibrium is obtained because of the piston or wall pressure of 2 atm. The net potential of the semipermeable container (O of solution minus piston pressure) is therefore less than the osmotic potential of its free solution by 2 atm. Since it is equivalent to the external solution with no wall pressure and an osmotic potential of 10 atm., we may call this net value the *osmotic equivalent* (E), so that

$$\text{osmotic equivalent} = \text{osmotic potential} - \text{wall pressure}$$

$$E = O - p \qquad (1)$$

The symbol E is particularly suitable, not only as an abbreviation for equivalent, but also to remind one that it is really a measurement of energy—osmotic potential energy; though instead of being measured in energy units, it is measured in terms of the maximum pressure that can be developed when the energy is converted into work. Thus E is really a measure of the water absorption energy (or potential) of the cell. Just as in electrical systems the emf is an energy unit that measures the work done in moving unit charge around a circuit, so in aqueous systems E, if it were expressed in energy units, would measure the work done in moving unit quantity of water across the semipermeable membrane. As the electric current is proportional to the emf when resistance is constant, so the current of water entering the cell is proportional to E when permeability (see below) is constant.

The osmotic equivalent of a system is defined as the osmotic potential of a solution at atmospheric pressure in equilibrium with it. This value can be determined experimentally by finding a solution of known osmotic potential that will neither withdraw water from nor give it up to the system.

Thus, if two solutions are under equal pressures, water will move from the one with the lower osmotic potential to the one

with the higher osmotic potential; but if the solutions are under different pressures, it will move from the system with the lower osmotic equivalent to the one with the higher osmotic equivalent.

Systems in osmotic equilibrium with each other must always have the same osmotic equivalents. But living cells are not in a condition of equilibrium as long as water is moving into or out of them, and it is frequently necessary to know in what direction and how rapidly water will move osmotically. Since the cells are normally under pressure (i.e., their walls are distended and they possess turgor), it is not enough to know their osmotic potentials; the water movement will always tend to be from the cell of lower osmotic equivalent (higher water activity) to the one of higher osmotic equivalent (lower water activity).

By analogy with the flow of electricity which is due to an electric potential difference, we may call this difference between the osmotic equivalents of two cells the *osmotic potential difference* though the direction of movement is opposite (from low to high osmotic equivalent). The rate of movement will at any instant depend on how large the osmotic potential difference is. It must be realized, however, that the osmotic potential difference P between two cells (1 and 2) is the difference between their osmotic equivalents and not simply their osmotic potentials, so that

$$P = E_1 - E_2 \tag{2}$$

$$= (O_1 - p_1) - (O_2 - p_2)$$

$$= O_1 - O_2 - p_1 + p_2 \tag{3}$$

On the other hand, the osmotic potential difference between a cell and the external solution (at atmospheric pressure) is

$$P = E_i - O_e \tag{4}$$

$$= O_i - p_i - O_e \tag{5}$$

where E_i = osmotic equivalent of the cell
O_i = osmotic potential of the cell
p_i = wall pressure of the cell
O_e = osmotic potential of the external solution

As in the case of the osmotic equivalent, the osmotic potential difference between two systems does not measure the energy directly but in terms of the maximum osmotic pressure that it can produce. Other terms such as *suction pressure*, *diffusion pressure deficit*, *turgor deficit*, etc. have been used for this quantity, but such terms have no true physicochemical meaning. Furthermore, they have been used indiscriminately for both the osmotic potential difference and the osmotic equivalent, and have therefore led to confusion.

From equations (4) and (5), the effect of each of the variables on osmosis can be readily determined: (a) If the external medium is water, O_e is zero and P becomes equal to E_i. Thus the osmotic potential difference between a cell and pure water is equal to the osmotic equivalent of the cell. (b) If equilibrium exists, P = zero and the osmotic equivalent of the cell is equal to the osmotic potential of the external solution. (c) If the external medium is water and equilibrium exists, both O_e and P are zero and therefore E_i is zero. Thus the osmotic potential of the cell is equal to the wall pressure ($O_i = p_i$). (d) If there is no pressure on the cell solution, p_i = zero and the osmotic potential difference is equal to the osmotic potential of the cell minus that of the external solution ($P = O_i - O_e$). (e) If equilibrium exists and there is no wall pressure (P and p are each zero), the osmotic potential of the cell equals that of the external solution ($O_i = O_e$).

Although the cell does not possess a piston, it is completely surrounded by a semipermeable membrane (at or just below the external surface of the protoplast) which is in its turn completely surrounded by a more or less rigid cell wall. It is this cell wall that takes the place of the piston in the system described above, and exerts a pressure on the protoplast. It must be realized that the pressures normally prevailing cannot squeeze the water out of the cell, as water is squeezed out of a sponge (applied pressures of 100–150 atm. are required to force water through the extremely fine "pores"); but it may cause water to diffuse out because it increases the free energy of the water molecules.

From Newton's third law of motion, it has long been known

that forces (and therefore pressures) come in pairs, the two being equal and opposite in direction. Thus the pressure exerted by the cell wall on the protoplast must be equalled by an opposite pressure exerted by the protoplast on the cell wall. Since this opposite pressure distends the wall and in this way gives rigidity (or turgor) to both the cell and the plant as a whole, it is known as *turgor pressure*. This pressure is due to water molecules in the protoplast impinging on the semipermeable membrane which transmits the pressure to the more rigid cell wall. It is therefore a hydrostatic pressure. If osmotic equilibrium does not exist, water will diffuse into or out of the cell, increasing or decreasing the (hydrostatic) turgor pressure, distending or relaxing the wall. If the distention of the wall is irreversible, the cell increases irreversibly in volume; that is, it grows.

Thus wall pressure affects the diffusion of water into and out of the cell, turgor pressure affects the rigidity and growth of the cell. These two are the only real pressures due to osmosis and may therefore be called osmotic pressures. No cell can develop a turgor pressure greater than the osmotic potential of the cell solution. In fact, the maximum turgor pressure is always less than the osmotic potential of a cell that is not fully turgid, because water must diffuse into the cell to increase the turgor pressure, and this causes dilution and a consequent decrease in osmotic potential.

When the osmotic quantities are varying, for instance, in the case of an expanding cell, the relationships are as shown in Fig. 15. Starting with a protoplast plasmolyzed to 0.9 of the cell wall volume, the osmotic potential is 16 atm., and so is the osmotic equivalent, since wall pressure is zero. When the protoplast has expanded sufficiently just to fill the cell wall, it has an osmotic potential and osmotic equivalent of 14.3 atm., and wall pressure is still zero. At a cell volume of 1.1, the osmotic potential has decreased still further (to 13.0 atm.) due to dilution of the cell contents by the entering water. The wall pressure has risen from zero to 3.2 atm. and the osmotic equivalent is 9.8 atm. (osmotic potential minus wall pressure). At full cell turgor (i.e., in equilibrium with pure water), the os-

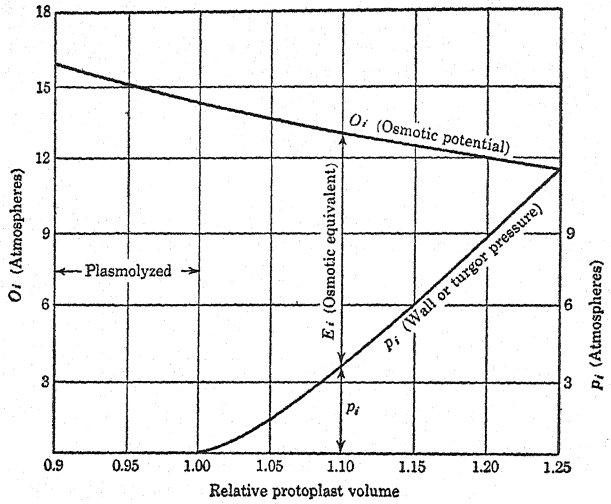


Fig. 15. Relations between osmotic equivalent E, osmotic potential O, and wall and turgor pressure p in an expanding protoplast and cell (parenchyma cells of sunflower petioles. (After Clark)

motic equivalent is zero, the osmotic potential and wall pressure are both 11.4 atm.

Determinations of osmotic quantities

Osmotic Potential. It is sometimes convenient to be able to calculate the osmotic potential of a solution from its concentration. This can be done by use of the following equation,

$$O = CRT$$

where O = osmotic potential in atm.
C = concentration in moles per liter
R = gas constant (0.082)
T = temperature in degrees absolute or Kelvin

If the solution is reasonably dilute and consists of monomolecular solute particles, this equation gives values that are sufficiently accurate for most physiologic work. If, however, the molecules dissociate into smaller particles as in the case of electrolytes (salts, acids, bases) the osmotic potential will be larger than calculated, by an amount equal to the excess of

particles over the expected number of molecules. In the case of NaCl, for instance, the right side of the equation has to be multiplied by 1.8, in the case of $CaCl_2$ by 2.4. This factor is known as the isotonic coefficient, and the equation becomes:

$$O = iCRT$$

where i is the isotonic coefficient (average number of particles per molecule).

Even in the case of some substances that do not dissociate, the first equation is not accurate if the molecules are hydrated to a considerable degree, e.g., in the case of sucrose. This error cannot be calculated for, since the degree of hydration is usually not known accurately enough and is not constant. A more nearly correct value can be obtained indirectly by determining the freezing-point lowering of the solution. Osmotic potential can be calculated from the equation $O = 12.06\Delta$ atm., where Δ is the freezing-point lowering as compared with pure water.

The osmotic quantities of cells and tissues cannot be calculated but must be determined experimentally. From (e), page 44, it can be seen that the osmotic potential of a cell may be determined by finding the solution that is just able to reduce wall pressure to zero when the osmotic potential difference is zero, i.e., at osmotic equilibrium. Wall pressure can be proved to be zero if no further contraction of the cell wall can be induced. This can be detected either by measuring the cell or more simply by observing for incipient plasmolysis, a barely detectable pulling away of the protoplasm from the wall, which can occur only when the wall is no longer distended. The osmotic potential of the external solution inducing this incipient plasmolysis can be calculated from the above equation or from the freezing-point lowering. This gives the osmotic potential of the cell. Since the cells are normally turgid, the values obtained from incipient plasmolysis are usually a little high.

If enough plant juice can be obtained, a second method is to determine its freezing-point lowering and calculate the osmotic potential as above.

Osmotic Equivalent. The method of determining this value is perhaps self-evident from its name. It is necessary simply to

find a solution in which no osmosis occurs either into or out of the cell. The osmotic potential of this solution is the osmotic equivalent of the cell. In practice, the cell or tissue strip is first measured, then immersed in solutions of different concentrations until one is found in which the cell or tissue strip neither increases nor decreases in size or weight.

The wall pressure and turgor pressure are difficult to determine directly and are usually calculated from equation (1).

Many measurements have been made of osmotic potentials of plant cells or juices. The values range from about 1 atm. to as high as 200 atm. Aquatic fresh-water plants are at the lower extreme, plants growing in soils of high salt content are at the upper extreme. It may be asked how a plant cell can withstand such high values, since a pressure of 1 atm. is about 15 lb. per in.^{2} But it must be realized first that these values are potential, not real, pressures (except in the case of aquatics and the roots of land plants where osmotic equilibrium or near equilibrium prevails). The only real pressures are the wall pressure and turgor pressure. It is the latter that may conceivably rupture the cell wall. A turgor pressure of 10 atm., for instance, means a pressure of 150 lb. per in.^{2} on the cell wall. But since the cell wall has such a small area, this may mean a very small force, e.g. 0.0003 lb. on the whole surface of a cell about 20μ in diameter.

REFERENCES

1. Clark, A. J. Personal communication.
2. Crafts, A. S., H. B. Currier, and C. R. Stocking. Water in the physiology of plants. Chronica Botanica, 1949.
3. Levitt, J. Toward a clearer concept of osmotic quantities in plant cells. Science, 113:228–231. 1951.
4. ———. The osmotic equivalent and osmotic potential difference of plant cells. Physiologia Plantarum, 4:446–448. 1951.
5. Scarth, G. W. and F. E. Lloyd. Elementary course in general physiology. Wiley. 1930.
6. Thoday, D. On the water relations of plant cells. Ann. Bot., 14:1–6. 1950.

Chapter 7

PERMEABILITY

The osmotic potential difference between a cell and its external solution controls not only the direction of movement of water but also the rate. But a reconsideration of the factors controlling rates of diffusion reveals that this does not take into account the permeability of the cell to the water. Of course, the statement that the cell is semipermeable implies that it offers no resistance to the movement of water. That this is not strictly true can be shown by allowing a collapsed but still living cell to expand in water. The wall expands ahead of the protoplasm (Fig. 16), showing that the cell wall is more permeable to water than is the protoplast.

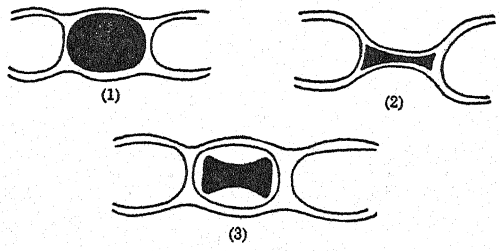


Fig. 16. Expansion of a collapsed (i.e., dried) living cell: (1) in water, (2) in air, (3) immediately after transfer from air to water. Final stage as in (1). (After Iljin)

The concept of semipermeability also implies that the solute cannot diffuse into the cell at all. This is true only in the case of some solutes. A great many others will penetrate the living cell, though usually less rapidly than water. In the case of all these solutes, the rate at which they enter the cell will depend not only on the concentrations of the solutes inside and outside the cell but also on the permeability of the cell to them.

Since the cell wall is a hydrophilic gel, diffusion of solutes

through it is usually unimpeded. This is evident from the fact that hypertonic aqueous solutions of all nontoxic solutes cause rapid plasmolysis of a living cell, as long as the cell is less permeable to the solute than to the solvent. Similarly, if dyes to which the protoplasm is normally impermeable (or nearly so) are injected into protoplasm, they diffuse freely and rapidly throughout the layer. Finally, there is nothing to prevent free diffusion of solutes throughout the cell sap, once they have entered the vacuole. But the same dyes that diffuse readily throughout the protoplasm fail to leave it either by diffusing out through the wall or into the vacuole. Thus the only impediments to free diffusion throughout the cell are the two protoplasmic surfaces, the so-called *plasma membranes*. The inner one is sometimes called the *tonoplast*. These surface layers differ from the main body of the protoplasm in their highly lipid (fatty) nature. As a result, they slow up and in many cases completely prevent the passage of solutes in either direction. Thus the plasma membranes are responsible for the semipermeability of the living cells.

A simple method of determining the permeability of cells to a solute is to plasmolyze them with a hypertonic solution of the substance. If the solute penetrates, deplasmolysis will occur, at a rate proportional to the permeability of the cell to the substance. Another method is to place the cell in a solution and after a definite time to analyze the cell sap quantitatively for the solute. This is most easily done with large-celled algae (e.g., species of Chara, Nitella, Valonia, etc.) from individual cells of which as much as a milliliter of sap can be removed. One of the most sensitive and rapid methods now available is to use a radioactive substance and to measure the increase in radioactivity of the cell sap or protoplasm. In this way it has been shown that radioactive P, Na, and K penetrate much more readily into the protoplasm than into the vacuole (Brooks).

By means of such methods, it can be shown that gases penetrate freely, all small molecules rapidly. This holds true as long as the molecular weight is not greater than 50–60 (e.g., water, ethyl alcohol, ethylene glycol). Electrolytes appear to be ex-

ceptions. Though in many cases their molecules are small, they penetrate slowly or not at all (e.g., NaOH). But it must be remembered that electrolytes dissociate into ions which are highly hydrated and therefore form rather large particles (larger than the undissociated molecules) since each ion must diffuse together with its shell of water molecules. The charge of the ions may perhaps also impede penetration. As a result, weak acids and bases penetrate more rapidly than strong acids and bases, because the former consist mainly of undissociated and therefore unhydrated (and uncharged) molecules. The solubility of the undissociated electrolytes in the membrane is also greater than that of the ions.

In the case of nonelectrolytes, each substance penetrates at a rate essentially independent of the presence of other substances. In the case of electrolytes this is far from true. Monovalent

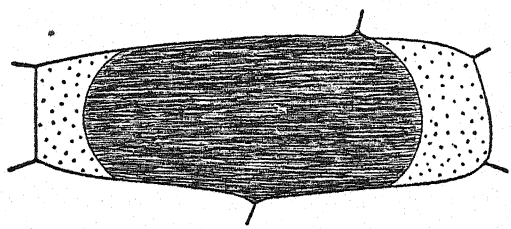


Fig. 17. Penetration of K into cytoplasm indicated by *vacuole contraction.* This is due to the increased hydrophily of the cytoplasm, causing movement of water from the vacuole (normally practically filling the cell—see Fig. 1) to the cytoplasm (normally an almost invisibly thin layer).

cations penetrate much more rapidly from single salt solutions than when a salt of a divalent cation is also present. In fact, single salt solutions may be toxic though the two-salt solutions are quite harmless. Thus a solution of KCl or NaCl or other salts of K and Na may cause swelling of the cytoplasm following rapid penetration, and *vacuole contraction* due to transfer of water from the vacuole to the swelling cytoplasm (Fig. 17.) This may eventually lead to injury or death. If, however, one-tenth as much $CaCl_2$ is added to the KCl or NaCl solution, no such extreme swelling occurs, and the cells may remain alive for days. This is because the $CaCl_2$ reduces or prevents the

penetration of the KCl or NaCl. Such effects of ions on each other are known as *antagonism*. The nontoxic solution of the two salts is called a *balanced solution*. It is interesting to note that, as mentioned above in the case of radioactive P, Na, and K, the KCl and NaCl penetrate the cytoplasm rather readily in single salt solutions, but do not seem to enter the vacuole. This indicates a difference in permeability between the outer and inner plasma membranes, at least for electrolytes.

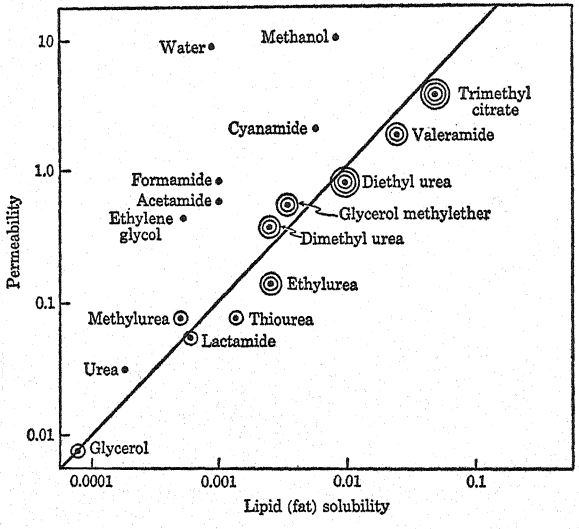


Fig. 18. Permeability of Chara cells to various organic nonelectrolytes. Molecular size indicated by the number of circles. (After Collander)

The permeability of cells to large molecules (i.e., with molecular weights above 50–60) varies from complete permeability (instantaneous penetration) to zero permeability, depending on the substance. Those large molecules that are lipid- (fat) soluble

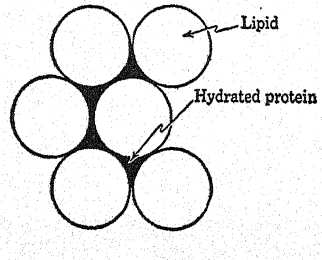


Fig. 19. Simplified diagram of the possible submicroscopic structure of the plasma membrane. (After Scarth and Lloyd)

penetrate rapidly, the more lipid-soluble the more rapid the penetration (Fig. 18). These results, taken in conjunction with the above-mentioned rapid penetration of small molecules that are not lipid-soluble, has led to the suggestion that the semi-permeable membrane is a lipid-sieve; that is, it consists of relatively large fatty particles separated from each other by much smaller aqueous pores (Fig. 19). Lipid-soluble molecules of any size would pass readily through the fatty particles, and only the smallest lipid-insoluble molecules would pass through the aqueous pores. But this concept is undoubtedly an over-simplification (see Davson and Danielli).

REFERENCES

1. Brooks, S. C. Penetration of radioactive isotopes, P^{32}, Na^{24}, and K^{42} into Nitella. J. Cell. and Comp. Physiol., **38**:83–93. 1951.
2. Collander, R. The permeability of plant protoplasts to small molecules. Physiol. Plant., **2**:300–311. 1949.
3. Davson, H. and J. F. Danielli. The permeability of natural membranes. Macmillan. 1943.
4. Iljin, W. S. Die Ursachen der Resistenz von Pflanzenzellen gegen Austrocknen. Protoplasma, **10**:379–414. 1930.
5. Scarth, G. W. and F. E. Lloyd. Elementary course in general physiology. Wiley, 1930.
6. Strugger, S. Praktikum der Zell- und Gewebephysiologie der Pflanze. Springer. 1949.

Chapter 8

ABSORPTION

Solutes

The absorption of solutes by living cells may sometimes follow the laws of diffusion and permeability already discussed. But there is a whole group of substances whose penetration into living cells cannot be accounted for on this basis, substances with molecular weights greater than 50–60 but that are insoluble in lipids. This includes many of the substances most important to the plant, i.e., sugars and many inorganic salts. That these substances do, indeed, fail to penetrate cells in sections of tissue is shown by the fact that one can plasmolyze the cells in solutions of dextrose or $CaCl_2$ and no deplasmolysis will occur even over a period of 24–48 hours. On the other hand, that they do penetrate the living cells of the unsectioned plant can be shown by feeding them to the roots or even by immersing tissue slices (e.g., potato tuber, carrot roots, etc.) in these solutions and bubbling air through the solutions. In periods of 24–48 hours very considerable amounts will go into the living cells. This absorption is something more than the result of diffusion and cell permeability.

Further evidence that absorption is not simply due to diffusion is the fact that it commonly occurs against a concentration gradient. But this may sometimes be more apparent than real. Thus living cells continue to accumulate dyes such as neutral red from a pale solution until the cell sap is intensely colored, due to adsorption of the dye by colloidal tannin particles in the vacuole. As a result, little of the dye remains in solution in the sap, and movement into the cell is according to the laws of diffusion, from a higher concentration outside to a lower concentration (in true solution) inside the cell. On the other hand, free ions are usually accumulated by living (and

particularly by actively growing) cells until they are present in much higher concentrations in the cell sap than in the surrounding medium (Table 9).

TABLE 9: RATIO OF SAP CONCENTRATION TO CONCENTRATION IN THE SURROUNDING MEDIUM [After Osterhout]

Ion	*Valonia macrophysa* (*in sea water*)	*Nitella clavata* (*in pond water*)
Cl	1.03	100.5
Na	0.18	46.1
K	41.6	1065.1
Ca	very small	13.2
Mg	very small	10.5
SO_4	0	25.8

The presence of large protein ions inside the protoplasm is capable of accounting for a moderate accumulation of individual ions in the cell. The large, negatively charged protein ions (anions) cannot pass through the plasma membrane, whereas the inorganic ions both of the same sign (anions) and of opposite sign (cations) can, at least under certain conditions. The protein anions inside the cell must be electrically balanced by enough cations to supply an equal number of opposite charges. If now the cell is bathed in a solution containing the same kind of cation as is inside the cell, plus an anion not found inside the cell (or present there in lower concentration), the new anion will tend to diffuse in. But electrical balance must be maintained; consequently for each anion that diffuses in, a cation of equal charge must also be dragged in. Simple diffusion equilibrium therefore cannot prevail and the cation will reach a concentration inside the cell greater than outside the cell. On the other hand, the anion diffusing into the cell will reach a concentration less than that outside the cell. This equilibrium that is controlled by electrical as well as diffusion phenomena is known as the *Donnan equilibrium.*

But the ion accumulation in plant cells is many times too great to be accounted for in this way, and furthermore there is a simultaneous accumulation of oppositely charged ions (Table 9, Nitella). Consequently, neither the kinetic (diffusion) energy nor the electric (Donnan equilibrium) energy of the ions is

capable of explaining this type of absorption. From the laws of thermodynamics, it therefore follows that some other kind of energy must be responsible for it. Since the only other source of energy available to the plant is from the respiratory process, it is not surprising that the rate of absorption against a concentration gradient is proportional to the respiratory rate (Table 10). Recent results have also shown that respiratory inhibitors prevent this kind of absorption and permit only that due to diffusion.

TABLE 10: ABSORPTION OF KBr FROM 0.00075 *N* SOLUTION BY CARROT DISCS
[After Steward, Berry, and Broyer]

Per cent O_2	*Relative respiration rate*	*Relative* K *absorption*	*Relative* Br *absorption*
2.7	43	22	42
12.2	78	96	86
20.8	100	100	100
43.4	106	117	118

Since substances can be absorbed in two different ways we can call the two *passive* and *active absorption*,* respectively. Passive absorption is due to diffusion and therefore follows diffusion or Donnan equilibrium gradients. Active absorption occurs against concentration (and Donnan equilibrium) gradients and at the expense of respiratory energy. It is because of this active absorption that the plant is able to accumulate large quantities of mineral nutrients from very weak solutions. If, however, the cells are deprived of O_2, not only are they unable to accumulate ions, but they will actually lose much of what they had previously accumulated. This shows that respiratory energy is needed not only to accumulate the ions but also to maintain them in the cells. Thus the plasma membrane may be visualized as a microsieve, through which the accumulated ions are constantly leaking out. Only if the living cell has a pumping system, capable of pumping the ions back into itself as rapidly as they are leaking out, can these high concentrations be maintained. This, however, probably applies only to the ions in the protoplasm. Those in the vacuole cannot be so easily lost.

It follows that in order for cells to grow and multiply (and in

* These two terms have also been used in a different sense (see p. 65).

many cases even to stay alive) they must possess the ability to absorb actively. Though most of the experimental work has used electrolytes, there is some evidence that this is also true of nonelectrolytes such as sugars, though recent work seems to indicate that they must first be converted to sugar phosphates before they can be absorbed (Street and Lowe). On the other hand, the living cell cannot actively absorb substances that are highly lipid-soluble, since it would require too much energy to do so; these substances would "leak" through the plasma membrane so rapidly that it would be like keeping a large-pored sieve full by pumping the water back as fast as it leaked out.

In spite of the fundamental importance of the process, the mechanism is not well understood. It seems to involve first an adsorption of the ion on the cell surface. This does not require respiratory energy, and can occur as rapidly at low temperatures (0°C.) as at normal growing temperatures. The adsorbed ions are then actively transported across the plasma membrane and into the protoplasm by a process linked with respiration, possibly due to the reversible oxidation and reduction of a respiratory enzyme containing ionizable iron (Lundegårdh). This active transport can occur only at temperatures normal for growth.

Water

As in the case of solutes, according to the laws of diffusion, water should enter a cell from any medium in which the water molecules have a higher activity (or free energy). Thus, in order for water to diffuse from the root medium through the root epidermis, cortex, endodermis, pericycle, and finally into the vessels, it must follow an osmotic gradient (Fig. 20). The living cells would affect such water movement by maintaining this osmotic gradient (due to the semipermeable plasma membranes) and by giving rise to wall pressure which affects the diffusion of the cell water. The osmotic potential difference would be represented by osmotic potential of vessels minus osmotic potential of external medium (since the vessels are nonliving cells and have no wall pressure). The hydrostatic pressure due to this difference is known as the *root pressure*.

But how can this root pressure be maintained? Since the vessels are nonliving cells, they do not possess semipermeable membranes. It might therefore be expected that the solutes in the vessels would diffuse through the walls into the walls of adjacent cells, and from cell wall to cell wall until they leaked out of the roots into the surrounding medium. This leakage is believed to be prevented by a hollow cylinder of cells, one row thick, surrounding the central stele (in which the vessels are located) and known as the *endodermis* (Fig. 20, G). The endodermal cells have lateral walls that are impermeable to water because of impregnation with fatty substances. Sometimes the fatty substances form a ribbon around the cell wall known as the *Casparian strip*. These endodermal cells are alive. Therefore in order to pass through them, substances would have to penetrate the semipermeable protoplasm, since they cannot leak through the lateral fatty cell wall unless they are fat-soluble. Even when these cells are plasmolyzed, the protoplasm remains attached to the lateral walls (Fig. 20).

A second factor that would oppose maintenance of root pressure is dilution of the vessel sap by the water entering the vessels. This would have to be opposed by more rapid absorp-

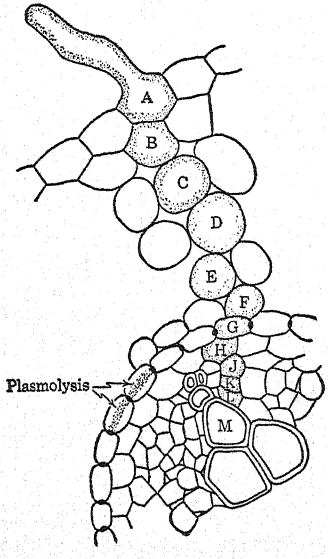


Fig. 20. Osmotic gradient in roots from root hair (cell A) to vessel (cell M). Water enters by moving along this gradient. Plasmolysis of two endodermal cells is shown to illustrate the strong adhesion of the protoplasts to the Casparian strips. (After Priestley)

tion of solutes than of water, in order to insure a higher concentration in the vessels than in the root medium. Or there might also be an actual secretion of solutes (e.g., salts or sugars) into the vessels from the adjacent living cells. Thus active absorption or active secretion of solutes must accompany absorption of water if root pressure is to be maintained.

Recent results have clearly demonstrated the need of oxygen for maximum water absorption by roots (Rosene). This is not surprising, in view of the need for active absorption of solutes in order to maintain root pressure. The active absorption of water itself is highly improbable except in very small amounts, since the water would "leak" out so rapidly by diffusion that enormous amounts of respiratory energy would be needed to maintain the gradient (Levitt).

Some aspects of root pressure remain to be explained. Though a diffusion gradient does normally exist between the external medium and the vessel sap (i.e., the vessel sap does have a higher osmotic potential than the soil solution) and though a cell to cell gradient has been found from the epidermis to the endodermis, yet the gradient apparently reverses itself from the endodermis to the vessel sap. Several explanations for this phenomenon have been suggested (e.g., active absorption of solutes by the endodermis) but experimental evidence is inconclusive.

REFERENCES

1. Levitt, J. Further remarks on the thermodynamics of active (nonosmotic) water absorption. Physiol. Plant., **6**:240–252. 1953.
2. Lundegårdh, H. Absorption, transport, and exudation of inorganic ions by the roots. Arkiv. f. Bot., 32A(**12**):1–139. 1945.
3. Osterhout, W. J. V. The absorption of electrolytes in large plant cells. I. Bot. Rev., **2**:283–315. 1936. II. Bot. Rev., **13**:194–215. 1947.
4. Priestly, J. H. The mechanism of root pressure. New Phyt., **19**:189–200. 1920.
5. Rosene, Hilda F. The effect of anoxia on water exchange and oxygen consumption of onion root tissues. Jour. Cell. and Comp. Physiol., **35**:179–193. 1950.

6. STEWARD, F. C., W. E. BERRY, and T. C. BROYER. The absorption and accumulation of solutes by living plant cells. Ann. Bot., 50:345–366. 1936.

7. STREET, H. E. and J. S. LOWE. The mechanism of sucrose absorption by excised roots. Ann. Bot., 14:307–329. 1950.

Chapter 9

ASCENT OF SAP

After the water has entered the roots, it rises to all parts of the plant. This process is known as the *ascent of sap*. Since diffusion is much too slow to account for the rates that commonly occur, a mass movement must be involved; the whole column must move simultaneously, instead of by molecular movement. Simple calculations reveal that the force required to lift the water to the top of the tallest trees is large. In the case of a 300-foot tree a pressure of 10 atm. is just sufficient to support the column of water; and in order to overcome frictional resistance, according to the available experimental evidence, about another 10 atm. is needed if the water is actually flowing through the tree. Since measurements of the root pressure of trees usually yield values of only 1–2 atm., the ascent of sap in tall trees cannot be due to root pressure. Some higher values have been obtained, e.g., 6 atm. in the case of tomato roots, and it is possible that the normal value may sometimes be higher than the commonly recorded values, though there is no good evidence of this.

The most conclusive evidence that the ascent of sap is not commonly due to root pressure is the fact that negative instead of positive pressures usually exist in the xylem (the water-transporting tissue). This is shown by the absorption of water through bore-holes in tree trunks. If such holes are connected by a siphon to an elevated bottle of water, as much as a gallon can be absorbed over a 24-hour period by an average-sized apple tree on a warm summer day. On the other hand, when root pressure is operative, sap exudes from such bore-holes. The only time such positive pressures occur in the xylem is when the leaves are not on the plant (in the case of deciduous plants) or when the plant is in a saturated or near saturated condition. In the latter case, water may actually be exuded

from the leaves. This phenomenon is known as *guttation* and it may be stopped by reducing the root pressure, for example, by watering the soil with a sugar or salt solution, thus increasing the osmotic potential of the root medium.

That the negative pressure (or tension) increases during the day can be shown by use of a sensitive instrument known as a *dendrograph*. This measures the diameter of the tree trunk and reveals a definite contraction during the day, an expansion at night (Fig. 21).

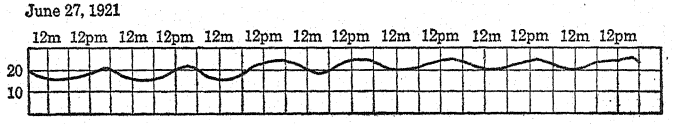


Fig. 21. Daily expansion and contraction of a redwood tree, amplified 10 times; m = noon, pm = midnight. (After MacDougal and Shreve)

Thus the ascent of sap is usually associated with a pull from above rather than a push from below. It is easy to calculate whether this can be explained by a capillary pull in the vessels, for

$$h = \frac{2S}{rdg}$$

where h = height (cm.) supported in the capillary tube
S = surface tension of liquid (dynes per cm.)
r = radius of tube (cm.)
d = density of liquid (g. per cm.^{2})
g = acceleration due to gravity (980 cm. per sec.^{2})

A vessel diameter of 0.1 mm (0.01 cm.) would account for only a 30 cm. rise. But it is not in the vessels themselves that the main capillary pull occurs. Since this is a surface tension phenomenon, the pull must occur at the water surface, and since the water system of a plant is continuous from the lowest roots to the highest leaves, the upper surface of the water column is in these leaves. It must, therefore, be at the outer surface of the leaf (mesophyll) cells that are in contact with the intercellular spaces, i.e., in the microcapillaries of their walls. Since

these microcapillaries are so fine that they cannot be seen with the ordinary optical microscope, their diameters must be less than 0.1 μ. Using this value in the above equation for capillary height, we find that these microcapillaries are capable of exerting sufficient pull to support a water column 300 meters high—three times the height of the tallest trees!

Unfortunately, physicists have not determined within what limits the above equation holds. Some go so far as to state that the rise is basically a push from below by atmospheric pressure and therefore cannot exceed 30 feet. But this concept is easily shown to be incorrect, since the capillary rise occurs just as readily in a vacuum. The classical Askenasy experiment has shown that the rise in purely physical systems can be two to three times that due to atmospheric pressure. But such experiments are difficult to perform and have not succeeded in duplicating the rise of hundreds of feet in tall trees. The theoretical concept is shown in Fig. 22. The following three stages in the process may be distinguished: (1) transpiration (the evaporation of water) from the surface of the microcapillaries; (2) cap-

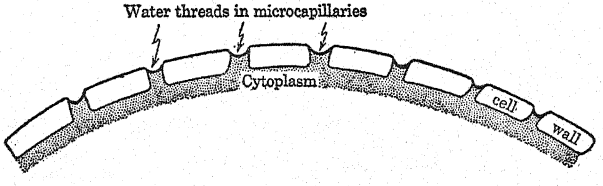


Fig. 22. Schematic diagram of the cross section of a mesophyll cell wall. For simplicity the capillaries are shown far apart, enlarged, and at right angles to the cell wall. The capillary pull occurs at the surface of the water threads.

illary rise of water threads due to the force of adhesion between the water and the cell wall (i.e., the force of imbibition); (3) the whole column of water moves en masse because of the force of cohesion between the water molecules. These three stages, of course, occur practically simultaneously. This concept is known as *Dixon's cohesion theory* of the ascent of sap, after the physiologist who first proposed it.

But even when it is stationary, a column of water 100 m. high

is subjected to a gravitational pull of 10 atm., and if the above reasoning is correct, this is counteracted by an upward surface tension pull of 10 atm. The column is therefore being subjected to a powerful force tending to break it. The question is whether a liquid such as water has sufficient tensile strength to resist the pull without rupture, and whether the force of attraction between the water and the vessel wall is sufficient to prevent separation from the wall. In other words, are the forces of cohesion and adhesion adequate?

Physical chemists have shown experimentally that pure water has a cohesive force of hundreds of atmospheres (theoretically about 1000). But the water in the vessels is not pure. Besides solids, gases are present in solution. The reduction in pressure due to the capillary pull may therefore reduce the solubility of these gases until they separate from the liquid, causing rupture of the column. Under conditions of excessive tension, vessels have in fact been found to become gas-filled. But since there are many columns of vessels side by side, it is not necessary for all of them to be continuous, and therefore the temporary filling of some vessels with gas may not be injurious. When the tension is relieved, by rain or simply at night, the gases go back into solution, and the column becomes continuous again.

Because of its relation to transpiration (or evaporation), the capillary pull is sometimes called *transpirational pull*. Thus the whole column is pulled upward en masse as though it were a solid wire. This pull would, of course, be still greater than the forces needed simply to support the column, since the moving column must also overcome the frictional resistance to movement. Since the column of water moves en masse, this means that the transpirational pull is transmitted from the microcapillaries in the mesophyll cell walls to the protoplasm and vacuole, to the adjacent cells, and to the nearest vessel. It is transmitted down the vessels through the leaf blade, petiole, stem, and all the way to the roots. From the base of the vessels in the roots the pull is transmitted through the adjacent cells to the epidermal cells and even to the medium surrounding these cells, i.e., solution or soil particles. Thus the tran-

spirational pull is responsible not only for the movement of water within the plant, but also for absorption of water from the root medium. When the transpirational pull is pronounced, it follows that diffusion plays little or no role in the absorption of water (Kramer).

This has led to use of the term *active* absorption for water absorption due to root pressure, and *passive* absorption for that due to transpirational pull. This use of the terms is very different from that of the previous chapter, and is less desirable, since *active* implies an association with respiration.

The mass movement of water under tension in the plant is rapid, as can be shown by cutting the stem of a wilted plant under water. Recovery occurs more rapidly than if the roots of the intact plant are watered. This illustrates the fact that though nearly the whole path of water movement is through dead vessels, the few living cells in the root offer a greater resistance to movement than do the vessels. This is no doubt due to the retarding effect of the semipermeable plasma membranes, which are considerably less permeable to water than are the cell walls. The resistance to flow in the vessels is due simply to the low frictional resistance or viscosity of the water itself.

Tensions in the vessels of up to 100 atm. have been reported. This points to the need of thick-walled cells, in order to prevent a collapse that would close the cavity, stopping the flow of water. The rate of water movement varies greatly, but speeds as high as 75 cm. per min. have been recorded (Huber). Since water movements occur only in a relatively narrow zone of the sapwood (the heartwood vessels are blocked by growths known as tyloses and by resins) it is here that the tension is localized. This means that when tensions are high, the vapor pressure of the water in the sapwood is markedly reduced below that in the heartwood. As a result, there is evidence that water distills from the heartwood to the sapwood on a hot summer day (Reynolds).

REFERENCES

1. Dixon, H. H. The transpiration stream. University of London Press. 1924.

2. HUBER, B. Beobachtung und Messung pflanzlicher Saftströme Ber. deut. bot. Ges., **50**:89–109. 1932.

3. KRAMER, P. J. Plant and soil water relationships. McGraw-Hill. 1949.

4. MACDOUGAL, D. T. and F. SHREVE. Growth in trees and massive organs of plants. Carnegie Inst. of Wash., Pub. 350. 1924.

5. REYNOLDS, E. S. Tree temperatures and thermostasy. Ann. Mo. Bot. Gard., **26**:165–255. 1939.

Chapter 10

TRANSLOCATION OF SOLUTES

Though the aim of physiology is to explain all living processes in terms of physics and chemistry, this goal has in most cases not yet been reached. As a result, it is frequently necessary to give a number of possible explanations (hypotheses) and to attempt to evaluate each on the basis of physical and chemical theory, together with the available physiological evidence. Sometimes it is possible in this way to exclude all the hypotheses except one. This has been done in the case of the ascent of sap. Thus, though several explanations have from time to time been proposed for the phenomenon, all except the cohesion theory have been conclusively disproved. In an elementary course there is no time to discuss these disproved theories, and the cohesion theory is accepted, not because it is conclusively proved, but because it and it alone agrees with the known facts. In the case of the translocation of solutes, however, the evidence does not permit acceptance of any one hypothesis. This has led to more controversy than in almost any other field of plant physiology. In the present state of uncertainty it is therefore necessary to examine more than one theory, together with the evidence on which each rests.

There are two principal translocatory systems in the plant, the xylem vessels and the phloem sieve-tubes. These two systems are quite different both morphologically and physiologically. The sieve-tubes are thin-walled, living cells, the vessels thick-walled, dead cells. Because they are alive and therefore semipermeable, though some workers believe their permeability is abnormally high (see Crafts), the sieve-tubes must possess turgor pressure. That this pressure is normally high is to be expected from the high osmotic potential of the sieve-tube sap (15–34 atm.) and from the fact that the cells are normally near full turgor due to the close proximity of the vessels with their

sap of low osmotic potential. Thus in a transpiring plant, though the vessels are slightly collapsed by the tension, the sieve-tubes may still be distended by their turgor pressure. As a result, a cut across the stele causes *exudation* ("bleeding") from the sieve-tubes, absorption by the vessels. Of course, a high rate of transpiration and relatively low rate of absorption by the plant may result in a sufficiently severe tension on the vessels to reduce the sieve-tube turgor to zero. Under these conditions, no exudation is possible.

Though the ascent of sap was long ago conclusively shown to occur in the xylem vessels and tracheids, the path of solute movement has been the subject of considerable controversy. According to the classical concept, mineral substances are carried through the xylem vessels by the ascending sap, but organic substances move in the phloem sieve-tubes. There is much indirect evidence for this view. Analysis of the xylem sap (obtained by centrifuging) reveals the presence of inorganic salts (Table 11). On the other hand, sieve-tube exudate consists mainly of sugars (Table 12). The concentration of total solutes

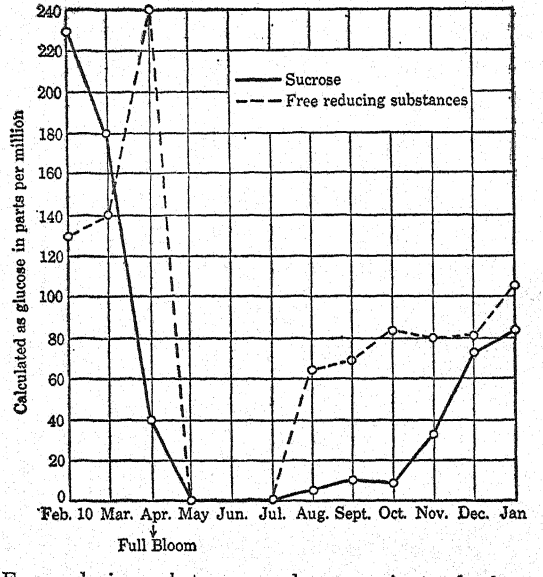


Fig. 23. Free reducing substances and sucrose in tracheal sap of pear branches. (After Anderssen)

may be 15–30 per cent, of which 50–90 per cent may be sucrose. Most of the rest is organic nitrogen, though Ca, Mg, and K may be present in significant amounts (Table 12) and P (though not Ca) has been found in the phloem exudate by Mason and Phillis. Sugar may reach a relatively high concentration in the xylem, at least during spring (Fig. 23).

TABLE 11: ANALYSIS OF TRACHEAL (VESSEL) SAP OF PEAR
[After Anderssen]

	Parts per million in sap	
Element	*Nov. 10*	*May 10*
Ca	16.6	84.7
Mg	0.8	23.5
K	23.6	59.6
Fe	1.0	2.1
SO_4	8.3	31.8
Cl	3.2	4.5
PO_4	10.6	25.2

TABLE 12: ANALYSIS OF PHLOEM EXUDATE OF ROBINIA PSEUDO-ACACIA DURING AUGUST [After Moose]

Element or substance	*mg. per cc.*
Ca	0.72
Mg	0.38
K	0.95
Li	trace
Reducing sugars	0.51
Sucrose	199.94
Total sugars	200.45
Nitrate nitrogen	0.135
Total nitrogen	0.56

These and many other apparently contradictory facts have led many physiologists to re-examine the classical concept of solute translocation. It was even suggested that all substances, both organic and inorganic, move in the xylem. But this concept has since been proved wrong. Thus Mason and Maskell showed by direct analyses that sugars and organic nitrogen move in the phloem. The usually rapid ascent of the xylem sap precludes the possibility of accounting for the normal downward movement of these solutes, which has been estimated to be at least 10 cm. per hr., with values as high as 100 cm. per hr. Curtis, on the other hand, has championed the phloem as the

main path of movement of all solutes, inorganic as well as organic. And it must be admitted that inorganic substances frequently do move in a direction opposite to the transpiration stream (see below). Nevertheless, whatever substances are in the xylem must be carried along in the ascending sap, so it is not possible to preclude the xylem as at least one path of movement for solutes, particularly the inorganic substances that always seem to be present in the xylem sap.

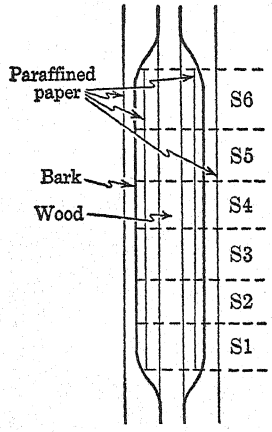
Most of the evidence has been obtained by ringing the stem or by separating the bark from the wood, or by cutting the wood. Such methods have consistently shown that both sugars and organic nitrogenous substances move in the phloem. If introduced into the transpiration stream they can, of course, move via the xylem, but this normally occurs primarily only in the spring. The general effects of ringing have long been known, e.g., the accumulation of carbohydrates above the ring, and such effects have been explained as due to movement of sugars in the phloem.

Similar methods have been used to follow the movement of inorganic substances, but with conflicting results. Mason, Maskell, and Phillis conclude that the xylem is the main path of movement of inorganic substances; Curtis concludes that it is the phloem. Curtis has suggested that the path of movement of soil substances may possibly depend on several factors, such as the quantity available. When this is high they may move in the xylem, when it is low they may move in the phloem. But there are other possible explanations for the differences obtained by these two groups of workers.

Recently, some conclusive evidence has been obtained by use of a research tool that has become available to the biologist only in the past decade and a half. This is the *radioisotope*, an element that emits radiations but has the same chemical properties as the normal, stable isotope. By means of these radiations, the element can be detected and measured quantitatively even when present in extremely minute amounts. With the aid of such substances, Stout and Hoagland conclusively showed that inorganic substances move up the plant in the xylem (Table 13). These results also revealed one source of difficulties. Whenever

bark and wood were left in normal contact, the substances moved readily into the phloem from the xylem. The substances in this way reached relatively high concentrations in the phloem both above and below the region of separation of bark from wood, sometimes even higher than in the adjacent xylem. Yet the extremely low concentrations in the separated phloem revealed that no movement occurred up or down the phloem other than that due to simple diffusion.

TABLE 13: UPWARD MOVEMENT OF RADIOISOTOPES FED TO THE ROOTS OF WILLOW AND GERANIUM (5 ml per l). Bark stripped from wood 1½ hours before feeding [After Stout and Hoagland]



	Willow (Gain of K, 5 hr. after feeding)			Geranium (Gain of PO_4, 6 hr. after feeding)	
	Bark	Wood		Bark	Wood
				270	860
			S8	9.0	112
	53	47	S7	0.5	120
S6	11.6	119	S6	0.6	132
S5	0.9	122	S5	0.8	138
S4	0.7	112	S4	< 0.3	147
S3	< 0.3	98	S3	< 0.5	137
S2	< 0.3	108	S2	< 0.3	152
S1	20	113	S1	11.1	131
	84	58		316	442

But even these experiments must be accepted with some reservation. As in all such techniques, injury to the bark is difficult to preclude completely, and their results do not prove that movement of these substances cannot also occur in the phloem. In fact, later results conclusively showed that they can. Thus Biddulph and Markle injected radiophosphorus into leaves of cotton plants having bark and wood separated as in Stout and Hoagland's experiment. In this case movement was

downward instead of upward. They found that it moved only via the phloem, and at rates in excess of 21 cm. per hour (Table 14). As in Stout and Hoagland's experiments, the radioisotope was able to move from one tissue to the other when the two were left in contact. These results also seem to disprove any suggestions of injury to the phloem in Stout and Hoagland's experiments. For if separation of the phloem from the xylem fails to prevent downward movement in the phloem, it is hardly likely to prevent upward movement.

TABLE 14: DOWNWARD MOVEMENT OF RADIOPHOSPHORUS INJECTED INTO A LEAF OF A COTTON PLANT. Leaf strip immersed in solution for 5 minutes, then in water. Bark stripped as in Table 13 [After Biddulph and Markle]

μg. PO_4 *in stripped stem after* 3 *hr.*		*μg.* PO_4 *in unstripped stem after 1 hr.*	
Bark	Wood	Bark	Wood
.0.904		.0.444	
0.684	0.004	0.160	0.055
0.544	0.000	0.103	0.063
0.120	0.019	0.055	0.018
0.123	0.002	0.026	0.007
0.160		0.152	

Without the help of radioisotopes, Mason, Phillis, and Maskell had earlier produced evidence that N, P, K, S, Mg, and Cl are phloem mobile in a downward direction, but that Ca is not. More recent results have confirmed this immobility of Ca in the phloem (see Arisz). Thus we are forced to admit that inorganic substances may move both in the xylem and the phloem; but the available evidence favors the view that upward movement of these substances is primarily, if not solely, in the xylem, downward movement probably solely in the phloem. Since by far the greater part of the movement of these substances is in the upward direction, this would assign to the xylem the main path of movement of inorganic substances, whereas the phloem is normally almost the sole path of movement of organic substances and the path of downward movement of all solutes. The classical concept is therefore reasonably near the truth.

As to the mechanism of movement in the phloem, two prin-

cipal theories have been proposed: (1) the mass flow, or more correctly *pressure flow* theory, since both theories imply a mass flow as opposed to a molecular (diffusion) movement (for arguments in its favor see Crafts), (2) the *cytoplasmic streaming* theory (for defense of this theory see Curtis and Clark). The first of these was proposed by Münch in 1926. He thinks of translocation in the plant as a kind of circulation analogous to that of the bloodstream, with the mesophyll cells in the leaves acting as a kind of heart. Water and salts move up the xylem, organic substances down the phloem, and an osmotic mechanism controls the latter (Fig. 24). As a result of photosynthesis, the osmotic potential of the mesophyll cells at the top of this circulatory system is maintained high, even though some solutes are being exported down the phloem. Due to the upward

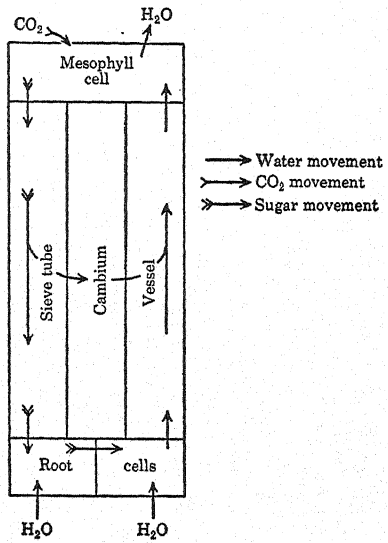


Fig. 24. Diagrammatic representation of pressure flow theory. (After Münch)

movement of water in the xylem, the water content is maintained at a high level. These two factors combine to yield a high turgor pressure in the mesophyll cells. Small pores known as pits occur in the cell wall through which cytoplasmic strands known as *plasmodesmata* connect these mesophyll cells with each other, and ultimately with the sieve-tubes. Through these

the turgor pressure succeeds in forcing some of the cell solution into and down the sieve tubes. The loss of solutes from the mesophyll cells would then be compensated for by newly synthesized organic substances, the loss of water by movement from the xylem. Excess water forced into the phloem stream would be "squeezed" laterally through the cambium into the xylem as the solutes are conducted downward. Movement in the phloem would then always have to be from a region of high to one of low turgor pressure. It could therefore occur in an upward direction from storage tissues. But at any one time, all substances would have to move in the phloem in the same direction. Movement would also depend on the existence of open plasmodesmata between all living cells. There are many other factors that have to be taken into account in evaluating the Münch "Druckström" theory. The strongest experimental evidence in its favor is the existence of exudation pressure in the phloem. That the Münch theory must at least be modified is indicated by the fact that the osmotic potential of the sieve-tubes is greater than that of the mesophyll cells (see Arisz).

The second theory was suggested by De Vries and in more recent years championed by Curtis. According to this concept, movement is due to a combination of diffusion and cytoplasmic streaming. Diffusion would occur from sieve-tube to sieve-tube across the intervening cell wall. Within the sieve-tube, the substances that have diffused in from the cell above would be carried downward by the cytoplasmic stream; those that diffused in from the cell below would be carried upward by the same cyclic stream. Thus this theory accounts for the known movement of substances along concentration gradients, and for the apparent fact that two substances can be simultaneously translocated in opposite directions (Mason and Phillis, Palmquist). By use of C^{14} and P^{32}, Chen has recently produced strong evidence of the simultaneous movement of carbohydrates and phosphates in opposite directions. But in view of results of Stout and Hoagland (Table 13), the P^{32} may have moved up in the vessels and laterally to the sieve-tubes. There is evidence from other sources that some organic substances can be translocated only in the same direction as sugars, for example, the

weed killer 2,4-D and viruses. Several other objections have been raised against the cytoplasmic streaming theory, though some at least are no longer valid. Thus it was stated that no streaming occurs in sieve-tubes. But careful technique has succeeded in demonstrating that it does occur (see Crafts, Curtis and Clark). Perhaps the most difficult point to reconcile with this theory is the fact that the phloem exudate is apparently cell sap and not cytoplasm, yet it contains high concentrations of the translocated substances (Table 10). This would seem to indicate that translocation is via the vacuole rather than the cytoplasm.

We are forced to conclude that neither theory is in full agreement with the facts, and a satisfactory explanation must await further evidence. Arisz suggests that this explanation may prove to possess elements of both theories.

REFERENCES

1. Anderssen, F. G. Some seasonal changes in the tracheal sap of pear and apricot trees. Plant Physiol., **4**:459–476. 1929.
2. Arisz, W. H. Transport of organic compounds. Ann. Rev. Pl. Physiol., **3**:109–130. 1952.
3. Biddulph, O. and J. Markle. Translocation of radiophosphorus in the phloem of the cotton plant. Am. J. Bot., **31**:65–70. 1944.
4. Chen, S. L. Simultaneous movement of P^{32} and C^{14} in opposite directions in phloem tissue. Am. J. Bot., **38**:203–211. 1951.
5. Crafts, A. S. Movement of assimilates, viruses, growth regulators, and chemical indicators in plants. Bot. Rev., **17**:203–284. 1951.
6. Curtis, O. F. The translocation of solutes in plants. McGraw-Hill. 1935.
7. Curtis, O. F. and D. G. Clark. Plant physiology. McGraw-Hill, 1950.
8. Mason, T. G. and E. Phillis. The migration of solutes. Bot. Rev., **3**:47–71. 1937.
9. Moose, C. A. Chemical and spectroscopic analysis of phloem exudate and parenchyma sap from several species of plants. Plant Physiol., **13**:365–380. 1938.

10. MÜNCH, E. Die Stoffbewegungen in der Pflanze. Gustav Fischer, Jena. 1930.

11. PALMQUIST, E. M. The path of fluorescein movement in the kidney bean, Phaseolus vulgaris. Am. J. Bot., **26**:665–667. 1939.

12. STOUT, P. R. and D. R. HOAGLAND. Upward and lateral movement of salt in certain plants as indicated by radioactive isotopes of potassium, sodium, and phosphorus absorbed by roots. Am. J. Bot., **26**:320–324. 1939.

Chapter 11

EXCHANGE OF GASES

Since protoplasm is freely permeable to gases, an exchange of CO_2, O_2, and water vapor may readily occur between living cells and their environment unless an impermeable barrier separates the two. Thus the fine absorbing roots obtain O_2 from the soil and rapidly use it in respiration, which must proceed at a rapid rate to supply the energy for growth, active absorption of solutes, etc. The CO_2 produced in respiration rapidly diffuses out of the cells into the surrounding soil. There are some plants that grow normally though the root medium is deficient in oxygen, because the O_2 is transferred to the roots from the aerial part through the intercellular spaces. This is particularly true of some aquatic plants whose intercellular spaces make up as much as 70 per cent of the volume of their tissues, as compared with much lower values (e.g., 20 per cent) in most land plants. There is also some exchange of CO_2, O_2, and water vapor between the stem and its environment. In woody plants this occurs primarily through openings known as *lenticels*, since the corky covering of the stems is relatively impermeable to these

TABLE 15: CO_2 AND O_2 CONTENT OF GAS IN TRUNKS OF PALO VERDE (PARKINSONIA MICROPHYLLA) [After MacDougal and Working]

Date	CO_2	O_2
Jan. 15–16, 1931	3.4%	15.3%
17–18	4.0	14.6
19–20	3.8	14.9
Mar. 3–4	8.5	13.8
5–6	8.8	13.7
7–8	9.4	13.1
9–10	10.7	12.9
11–12	12.0	12.6
Apr. 20–21	7.3	14.5
22–23	7.8	15.2
23–24	9.1	14.3
25–26	9.0	14.6

gases. As a result, the gas in the trunk may be of very different composition from the air (Table 15). But the organ responsible for the major gas exchange of the plant is the leaf, and since most leaves are covered by a layer of relatively impermeable cuticle, the main exchange is usually through the *stomata*. These consist of two guard cells that impede gas exchange when tightly pressed together but permit free exchange when separated by a pore.

Stomatal movement

In general, the stomata tend to show a diurnal periodicity, closing at night and opening during the day (Fig. 25). That this periodicity is related to light is easily shown. Yet there are many exceptions (Loftfield). Some stomata open at night,

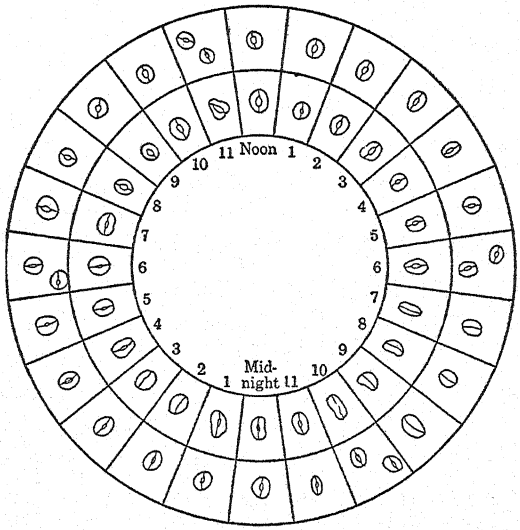


Fig. 25. Stomatal opening in alfalfa at different times of the day. Inner circle represents lower leaf surface; outer circle upper leaf surface. (After Loftfield)

others close at noon when the light intensity is at a maximum. Wilting may cause closure regardless of the light factor. Thus the effect of light on stomatal opening is apparently indirect, through its control of some other factor. In order to understand

the action of such indirect factors, the mechanism of stomatal movement must first be looked into.

Because of the uneven thickening of the walls of the guard cells, increased turgor leads to opening, decreased turgor leads to closure (Fig. 26). Since turgor is a hydrostatic pressure, the stomatal movement is controlled by water exchange, and any factor that alters the water content of the guard cells will affect their turgor and therefore the stomatal opening. The maximum possible turgor pressure of a cell is practically equal to the osmotic potential of the cell sap. Any increase in the osmotic

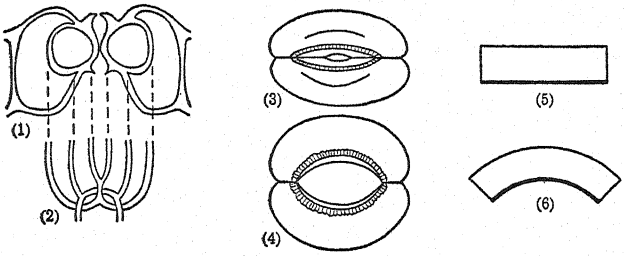


Fig. 26. Effect of changes in turgor pressure of guard cells on stomatal opening: (1) and (2), cross section and half surface view of stoma of *Amaryllis formosissima;* (3) and (4), surface views of closed and open stoma; (5) and (6), diagrammatic scheme of turgor curvature of guard cell when inner wall is thicker than outer wall. (After Benecke-Jost)

potential due to an increase in solutes will therefore favor opening, any decrease will favor closure. But it must be remembered that this change in solutes affects only the potential, and the actual change in turgor pressure can occur only as a result of water movement into or out of the cell. Any real change in turgor pressure, on the other hand, must be accompanied by a change in degree of opening, provided the cell wall is capable of a further stretch or shrinkage. But there is another factor. The other epidermal cells, as long as they are turgid, exert a back pressure on the guard cells; and if all the epidermal cells were to increase equally in osmotic potential and turgor pressure, no stomatal opening would result. Similarly if all decreased equally in osmotic potential and turgor pressure, no closure could occur. In fact a simultaneous water loss from all

the epidermal cells may result in stomatal opening if the guard cells lose less than the other epidermal cells. For this reason, incipient wilting may actually cause increased stomatal opening, though more pronounced wilting will cause closure. This is because the guard cells are the last of the epidermal cells to reach zero turgor pressure. Finally, of course, excessive loss of water will cause stomatal opening due to collapse of the guard cells.

The importance of the osmotic potential of the guard cells in stomatal movement is shown by the high values (20–100 atm.) that have been recorded for open stomata, and the low values (5–10 atm.) in the case of closed stomata. Theoretically, there are three possible causes of such changes (and there is some experimental evidence for each): (a) accumulation of sugars due to photosynthesis; (b) starch $\rightleftharpoons$ sugar transformations (starch $\rightarrow$ sugar leading to opening, sugar $\rightarrow$ starch leading to closure); (c) active absorption of solutes from surrounding cells. In monocotyledons (e.g., onion), photosynthesis leads to an accumulation of sugars. That photosynthesis occurs in the guard cells has been indicated by recent results. Consequently (a) may explain stomatal movement in monocotyledons if the sugars disappear from the guard cells in the dark. This has yet to be shown. In dicotyledons, photosynthesis usually leads to an accumulation of starch, and this does not increase the osmotic potential, since starch is insoluble. But the guard cells in these dicotyledons behave differently from the other leaf cells. In the daytime, the guard cells accumulate sugars like the cells of monocotyledons; at night, the sugars are converted to starch. All the other green cells of the dicotyledon leaf accumulate starch in the daytime and convert it at night to sugars which are then translocated to other parts of the plant. Therefore (b) controls the osmotic potentials of the guard cells of many dicotyledons. In these cases, the starch $\rightleftharpoons$ sugar conversion can be controlled by changing the pH of the guard cells. At high pH (about 7.0–8.0), starch is converted to sugar, and the stomata open. At low pH the reverse happens. These facts, together with many others, have led to the following theory of the mechanism of stomatal movement in many dicotyledons

(Scarth): (1) when the plant is exposed to light, photosynthesis occurs, and as a result, (2) the CO_2 content of the leaf is reduced; (3) the *p*H of the cells rises; (4) starch is converted to sugar in the guard cells; (5) the osmotic potential of the guard cells rises; (6) water enters these cells; (7) the turgor pressure rises; (8) the guard cells are forced apart, opening the stomata. Of these steps, the only one that is not self-explanatory is the effect of *p*H on starch $\rightleftharpoons$ sugar conversion. As will be seen when the metabolism of the plant is discussed, this is an enzymatically controlled reaction, and enzymes are usually active at some *p*H, and inactive at others. Recent results have indicated that the enzyme capable of converting sugar to starch (phosphorylase) is present in the guard cells. In the presence of this enzyme at *p*H 5, the ratio of starch to sugar (phosphate) is $3\frac{1}{2}$ times what it is at *p*H 7 (Peat). Recent results by Scarth and co-workers indicate that the starch to sugar transformation is not always able to account for the increased osmotic potential associated with stomatal opening. They believe that active absorption of sugars by guard cells from surrounding cells may also play a part.

Further evidence in favor of this theory is the recently dis-

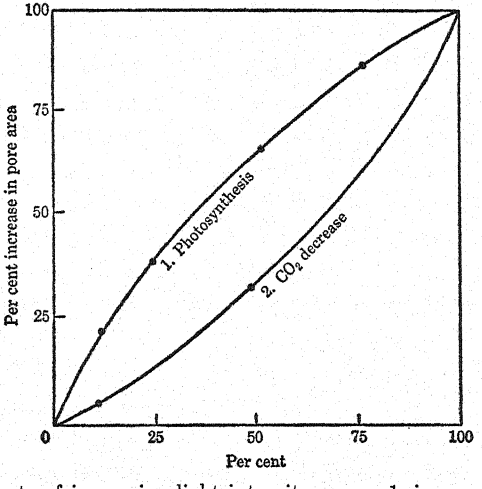


Fig. 27. Effects of increasing light intensity: curve 1, increased stomatal area accompanying increased rate of photosynthesis; curve 2, consequent decrease in CO_2 content of surrounding air. (After Scarth and Shaw)

covered fact that when the CO_2 content of the intercellular spaces is reduced from the 0.03 per cent in air to 0.01 per cent, the stomata open (Heath and Milthorpe). The close relation between rate of photosynthesis, CO_2 concentration, and stomatal opening is shown in Fig. 27. On the other hand, the behavior of the stomata is not always easily explained by this theory. Thus the night opening of the stomata cannot be related either to photosynthesis or to a reduction in CO_2 content. But it has been shown that an oxygen deficiency may also cause stomata to open, and it has been suggested that this results from an increase in *p*H that apparently may sometimes occur due to the absence of oxygen.

Diffusion of gases through small openings

The rapid rate of diffusion of gases into and out of the leaf is understandable on the basis of Fick's law of diffusion: the diffusion constant of gases is high (D for water vapor is about 10,000 times D for liquid water), the area across which diffusion is occurring is large, the concentration difference is large, and the distance is small. But the area is large only if we consider the whole leaf area. When, however, it is remembered that most of the gas movement occurs through the open stomata, the high rate of diffusion may be questioned, for the area of the stomata is only about 1 per cent of the leaf area. Yet a leaf may lose almost as much water as a free water surface of the same total area. This means that the rate of water loss per unit stomatal area is of the order of almost 100 times that from a free water surface. It has also been shown that a photosynthesizing leaf absorbs CO_2 about as rapidly as a KOH solution of the same total surface. One must therefore conclude that either the laws of diffusion must be modified or else some other process speeds up the diffusion from the stomata. Brown and Escombe settled this problem by showing that the loss of water from a free water surface is practically unaltered when it is covered by a perforated sheet, even though the area across which diffusion occurs is reduced to a small fraction. This can be explained by the overlapping of the diffusion fronts (Fig. 28). The molecules passing through the pores at the perimeter can diffuse more

freely in all directions due to the absence of other interfering molecules. They therefore spread out laterally and soon meet with similar molecules from adjacent pores, forming a solid front a short distance above the pores. Expressed quantitatively, this means that the diffusion across small openings is proportional to the perimeter rather than the area of the opening.

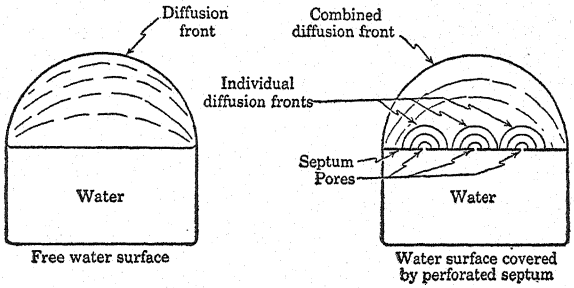


Fig. 28. Overlapping of diffusion fronts from apertures in membrane (or septum) covering a water surface.

Since the perimeter of a circle $= 2\pi r$, whereas the area $= \pi r^2$, in the former case diffusion is proportional to r, and in the latter case diffusion is proportional to r^2.

But it must be understood that as soon as overlapping occurs, there can be no further speeding up. Thus if the openings are as close together as 10–20 times their diameters, maximum diffusion occurs. Doubling the diameter of these openings will have no effect on the diffusion rate. This fact must be taken into consideration when explaining the control of gas exchange by stomatal movement.

Transpiration

Since the quantitative aspects of O_2 and CO_2 exchange have been investigated primarily from the point of view of respiration and photosynthesis and will be considered under these headings, water vapor is the only gas that will be dealt with at this point. The loss of water vapor from the plant has long attracted the attention of physiologists and has been given the name *transpiration.* As a general rule, transpiration is higher when the stomata are open, lower when they are closed. In

other words, the *stomatal transpiration* is usually higher than the *cuticular transpiration*, commonly about 4 times as high during a sunny day. But there are all gradations from one extreme to the other. In the case of succulents, cuticular transpiration is essentially zero. A joint of a prickly pear can be kept for several years without losing all its water. On the other hand, a shade plant (such as wood sorrel) may lose as much water through the epidermal cells as through the open stomata, because the cuticle layer is practically nonexistent.

Many early workers believed that stomatal movement completely controlled transpiration. But this was soon found to be untrue. Transpiration rate may actually increase when stomata begin to close, and may decrease when they open wider. This is sometimes due to the fact that when the diameters of the stomatal openings are one-tenth to one-twentieth their distance apart, no further opening can affect the rate of diffusion through them. But it may also be due to the one other variable factor that always controls the rate of diffusion, the concentration difference ($C_1 - C_2$ in Fick's law), or in the case of transpiration, the vapor pressure difference. The larger the difference between the vapor pressure of the leaf and that of the surrounding air, the greater the transpiration rate. Both the relative humidity and the temperature may affect the vapor pressure of the air and the leaf. Consequently, they may both alter the transpiration rate.

Wind may also affect the transpiration rate. It may do this by removing the saturated air layer in contact with the leaf, thereby steepening the vapor pressure gradient (i.e., reducing the distance across which the vapor pressure difference exists). But this will primarily affect the cuticular transpiration. The stomatal transpiration will be relatively slightly affected in this way, because the distance (x in Fick's equation) is not simply from one side of the cuticle to the other, as in the case of cuticular transpiration, but from the mesophyll cells across the intercellular spaces and stomatal cavity to the external surface of the leaf. This has been compared to the relatively slight effect of a wind outside an open window on the rate of evaporation from a beaker of water inside the room. But wind may markedly

affect transpiration rate, even though it has a relatively small effect on diffusion. This is because it may move the leaves and actually shake the water vapor out of the intercellular spaces. On the other hand, wind may sometimes actually reduce transpiration rate if it causes closure of the stomata.

There has been some controversy as to whether transpiration is a necessary evil or whether it is in some way essential for the welfare of the plant. From the former point of view, the function of the stomata is to permit exchange of CO_2 and O_2 in the processes of photosynthesis and respiration. At the same time water is lost through the open stomata because there is no way of stopping the passage of water vapor while permitting the CO_2 and O_2 exchange. From the other point of view, transpiration fulfills three main functions:

1. *Transpiration helps maintain optimum turgidity.* When a land plant is grown in a saturated atmosphere, it develops a softer, more watery type of growth. This is presumably due to the larger turgor pressure, causing excessive cell enlargement. The larger cells will also have thinner walls due to the excessive stretch. Aquatic plants avoid this danger by having low osmotic potentials (3–5 atm. in many cases of fresh-water plants). Terrestrial plants avoid it because they are seldom at or near full turgor; for even though their osmotic potentials may be high (up to 200 atm. in the case of some halophytes) transpirational loss keeps the turgor pressure well below the osmotic potential.

2. *Transpiration reduces the leaf temperature.* In full sunlight on a hot day, a leaf's temperature may be in danger of rising to the killing point. The evaporation of water is a cooling process and may help to prevent such heat injury. It has been shown that the actual cooling effect of transpiration is not so great as some workers have thought. But even though it may only cool the leaf 3°–5°C (Curtis), this may conceivably, in some cases, save the leaf from heat killing.

3. *It promotes the translocation of solutes.* This factor has been the subject of the most controversy. As already seen, the mineral elements absorbed from the soil are translocated mainly, if not solely, in the vessels of the xylem, rising in the

transpiration stream. The question is sometimes raised whether or not the speed of translocation of these solutes depends on the rate of transpiration. By analogy with an endless belt delivering coal, we can see that the rate of delivery depends not on the speed of rotation of the endless belt, but on the rate of transfer to it (Fig. 29). If substances enter the plant by active absorption, the amount reaching the leaves per unit time will depend, not on the speed of the transpiration stream (or of the endless belt) but on the rate of absorption (or of fall of the coal). On the other hand if we consider individual branches of a plant coming off the main transpiration stream, the one with the

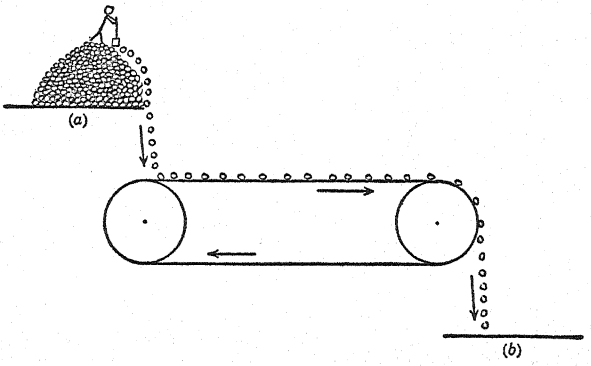


Fig. 29. Effect of transpiration on rate of translocation of solutes in xylem: analogy with an endless belt delivering coal; rate of delivery to (b) depends on rate of drop from (a), regardless of rate of movement of endless belt.

more rapid branch stream will tap off more from the main stream. Consequently, we might expect a more rapidly transpiring branch of a plant to receive a larger fraction of the absorbed nutrients.

If, however, the transpiration (or endless belt) is so slowed down that the solutes accumulate in roots (or the coal piles up at the beginning of the belt), the absorption rate will be stopped or at least greatly retarded. Many investigators have actually found that increased transpiration does increase the amount of substances getting into the plant from the root medium. And some feel that the above indirect effect on the absorption is inadequate to explain the results. It has been

suggested (Hylmö) that the increased tension produced by higher transpiration rates pulls the external solution through the intercellular spaces along the cell walls of the epidermal and cortical cells, in this way by-passing the more resistant semipermeable membranes of the protoplasm in all these cells. It would then be necessary for the solution to pass through the protoplasm of only one layer of cells, the endodermis, before being pulled into the xylem. Plausible as this may sound in the case of plants artificially grown in solutions (as in Hylmö's experiments) it cannot apply to roots growing in soil, where the solution is so weak that most of the solute absorption must begin by adsorption on the root surface; and such adsorbed substances could not be carried along bodily through the cell walls.

These interactions between transpiration and absorption point to an important principle. Numerous physiological processes occur simultaneously. Therefore, no matter how much we learn about each by studying it independently, it must ultimately be investigated when under the influence of the others, in order to understand it fully. This principle will be returned to again and again.

The actual rate of transpiration may be measured in several ways. The water given off may be collected in drying towers (e.g., by a desiccant such as $CaCl_2$) and the increase in weight of the towers will be equal to the weight of water lost. But this method requires enclosing the plants, which is difficult without altering the transpiration rate. Other methods are also available, but the simplest is to weigh a potted plant at intervals. The loss of weight is essentially equal to the loss of water from the plant, since other factors (e.g., carbon assimilation) usually have a negligible effect on the weight compared to the effect of water loss.

REFERENCES

1. Benecke, W. and L. Jost. Pflanzenphysiologie I. Jena. G. Fischer. 1924.
2. Brown, H. T. and F. Escombe. Static diffusion of gases and liquids in relation to the assimilation of carbon and trans-

location in plants. Phil. Trans. Roy. Soc. (London), **B 193**:223–291. 1900.

3. Curtis, O. F. Wallace and Clum, "Leaf temperatures": A critical analysis with additional data. Am. J. Bot., **25**:761–771. 1938.
4. Heath, O. V. S. and F. L. Milthorpe. Studies in stomatal behavior. V. The role of carbon dioxide in the light response of stomata. J. Exp. Bot., **1**:227–243. 1950.
5. Hylmö, B. Transpiration and ion absorption. Physiol. Plant., **6**:333–405. 1953.
6. Loftfield, J. V. G. The behavior of stomata. Carnegie Inst. Wash. Pub. 314. 1921.
7. MacDougal, D. T. and Earl B. Working. The pneumatic system of plants, especially trees. Carnegie Inst. of Wash. Pub. 441. 1933.
8. Peat, S. Biological transformations of starch. Adv. in Enzym., **11**:339–375. 1951.
9. Scarth, G. W. Mechanism of the action of light and other factors on stomatal movement. Plant Physiol., **7**:481–504. 1932.
10. Scarth, G. W. and Michael Shaw. Stomatal movement and photosynthesis in Pelargonium. Plant Physiol., **26**:207–225. 1951.
11. Yin, H. C. and Y. T. Tung. Phosphorylase in guard cells. Science, **108**:87–88. 1948.

Part III

PLANT BIOCHEMISTRY

Chapter 12

NUTRITION

The nutrition of any organism includes all the substances that must be supplied to it from the outside. Those organisms that require both organic and inorganic substances are called *heterotrophic;* those that need be supplied only with the inorganic substances are called *autotrophic* ("self-feeding" since they make their own organic substances). Animals and nongreen plants are heterotrophic. When nongreen parts (e.g., roots, stem tips, embryos) are *excised* (severed) from the green parts of the plant, they can be made to grow only when supplied with at least a sugar, and in many cases several other organic substances (Table 16).

TABLE 16: WHITE'S NUTRIENT MEDIUM FOR GROWING EXCISED ROOTS

Substance	*mg. per liter*
$MgSO_4$	360.0
$Ca(NO_3)_2$	200.0
Na_2SO_4	200.0
KNO_3	80.0
KCl	65.0
$NaH_2PO_4 \cdot H_2O$	16.5
$Fe_2(SO_4)_3$	2.5
$MnSO_4$	4.5
$ZnSO_4$	1.5
H_3BO_3	1.5
KI	0.75
Sucrose	2000.0
Glycine	3.0
Nicotinic acid	0.5
Pyridoxine	0.1
Thiamine	0.1

The normal, green plant, on the other hand, is autotrophic: it can synthesize all its essential organic substances, provided it is supplied with all the essential inorganic elements and grown under normal conditions (e.g., in the light). The nutri-

tion of green plants is therefore solely inorganic. It is, in fact, commonly called *mineral nutrition,* and the elements absorbed by the roots (excepting C, H, O) are called mineral elements, since they are mainly obtained either directly or indirectly from the minerals in the soil. The mineral nutrition of plants therefore embraces all the elements essential to the plant's existence, with the exception of C, H, and O. Nitrogen is, strictly speaking, not a mineral element but it is included with these since it is obtained by the plant from the soil.

The first and perhaps most fundamental problem of mineral nutrition is to determine which elements are essential to life and growth. As a start, one might analyze the plant to find out which are present. When this is done, the number of elements found depends on the plant, the medium in which it was grown, and the completeness of the analysis (Table 17). But this tells us merely that the elements not found in the plant (assuming that the methods of analysis are adequate) are not essential in appreciable quantities. The mere presence of an element in a plant does not prove its essentiality. Thus if a large enough number of plants from different regions were analyzed, certain elements might sometimes be present, sometimes absent from the plant. Actually, some 60 elements have been found in plants (Robinson and Edgington), and undoubtedly all the rest of the 90-odd known elements could be made to occur in the plant by supplying them to the roots.

A more direct approach to the problem of essentiality is to grow plants in the complete absence of a given element. If the plant grows normally, the element is evidently not essential; if it fails to grow normally, the element is usually considered essential. In order to be certain that the element is truly essential, it is necessary to show not only (1) that a deficiency of the element makes it impossible for a plant to complete its vegetative or reproductive cycle, but also that (2) it cannot be replaced by any other element, and (3) that the effect is not simply the result of interaction with (e.g., detoxification of) other elements, organisms, etc. These three requirements have been called the *criteria of essentiality* (Arnon and Stout). However, it has recently been shown that some of the accepted

essential elements can at least be partially replaced by others (e.g., Mg by Mn, K by Rb, etc.—Hewitt).

The search for essential elements has required the development of a technique for growing the plant in the absence of impurities. This has meant elimination of the natural root medium, the soil. In its place, a pure sand, gravel, or best of all, simple solutions have been used. The latter, so-called *water* or *solution cultures*, offer the greatest difficulty, since they fail to supply the normal support for the roots and the normal amounts of oxygen. This requires development of adequate artificial support and aeration. A successful water or solution culture therefore requires a certain amount of engineering. This system of growing plants in the absence of soil has been used to some extent on a practical scale, and has been named *hydroponics* (Gericke).

TABLE 17: ANALYSIS OF STEMS, LEAVES, COB, GRAIN, AND ROOTS OF CORN [After Miller]

Element	*Per cent of total dry weight*
O	44.4
C	43.6
H	6.2
N	1.46
P	0.2
K	0.92
Ca	0.23
Mg	0.18
S	0.17
Fe	0.08
Si	1.17
Al	0.11
Cl	0.14
Mn	0.035
Undetermined	0.93

Using only the first criterion of essentiality (i.e., no normal growth in the absence of the element) the following seven elements were long ago found to be essential: N, P, K, Ca, Mg, S, and Fe. Thus it was discovered that plants grow normally in four-salt solutions containing KNO_3, KH_2PO_4, $CaCl_2$, and $MgSO_4$ (or other salts supplying these six elements) with a small amount of $FeCl_3$ (or other iron salt). Even 3-salt solutions

(KH_2PO_4, $Ca(NO_3)_2$, $MgSO_4$) plus some iron were soon found adequate. Many formulas for such solutions have been adopted, some of them named after the workers who developed them, e.g., Knop's, Cronin's, Tottingham's, Shive's. But the early solutions were made with salts that were not pure. Small quantities of other salts were therefore invariably present. Since it was already shown that one element (iron) was needed only in a small quantity, it was logical to suspect that other elements might also be essential in trace amounts. It was soon found that the better the purification of the basic three or four salts the poorer the growth. This was followed up by direct evidence that other elements are needed in small amounts. They are just as essential for the life and growth of the plants as the above seven elements, and therefore no growth is possible in their complete absence. But in view of the small amounts needed, they are usually grouped (with iron) separately from the above six *major* or *macronutrient elements*, and called *trace*, *minor*, or *micronutrient elements*. So far, the following six have been conclusively proved to be trace essential elements: Fe, Mn, Cu, Zn, B, and Mo. They are needed in the nutrient solution in amounts ranging from 0.5 ppm. to 0.01 ppm. (Table 18). Larger quantities may cause injury or death. Some investigators have produced evidence for the essentiality of a few other elements (e.g., Al, Si, Se) at least for certain plants, but other workers have failed to confirm their results. Elements such as Al and Si have been called *ballast elements* (Frey-Wyssling) because they are normally present in large amounts, though the plant can be grown perfectly normally without them (as far as

TABLE 18: QUANTITIES OF MINERAL ELEMENTS USED IN COMPLETE NUTRIENT SOLUTIONS [After Robbins]

Major ion	*Quantities (moles)*	*Trace element*	*Quantities (ppm.)*
NO_3^-	0.005–0.010	Fe	0.5
PO_4^{---}	0.00025–0.002	B	0.25 (or more)
SO_4^{--}	0.001–0.010	Mn	0.25
K^+	0.002–0.010	Zn	0.25
Ca^{++}	0.002–0.005	Cu	0.02
Mg^{++}	0.001–0.010	Mo	0.01

can be determined). Some plants are *accumulators;* i.e., they concentrate large quantities of certain elements (e.g., Al) in their tissues.

Complete absence of any one of the essential elements (major or trace) will completely stop growth. But under normal conditions the elements are never completely absent. If, however, they are present in less than optimum quantities, growth will occur but abnormalities known as *deficiency diseases* will develop. The symptoms of such diseases are more or less specific for each element, though it is sometimes difficult to distinguish the differences. Furthermore, different plants will show somewhat different deficiency symptoms. Some occur first on the oldest leaves (e.g., N, K, P, Mg), others first on the youngest leaves (S, Ca, Fe, Mn, B, Cu, Zn). Elements of the first group are mobile and are transferred from the older inactive leaves to the younger growing leaves. The elements of the second group are immobile and remain in the old leaves, causing the newly developing leaves to show the deficiency though the older ones do not (Müller).

The commonest type of deficiency symptom is *chlorosis*, reduction in the amount of green pigment in the leaf. But chlorosis can occur as a result of any one of several deficiencies (N, Mg, Fe, Mn, etc.). However, the type of chlorosis is sometimes different for different elements. Thus it is a uniform loss of color in the case of N deficiency, but more pronounced between the veins in the case of Fe deficiency. The trace elements have given rise to a particularly large number of deficiency diseases, e.g., celery crack, heart rot and dry rot of sugar beets, internal browning of cauliflower (B deficiencies); little leaf or rosette of fruit trees (Zn deficiency); marsh spot of pea seeds (Mn deficiency), etc.

The deficiencies found in the plant may not correspond with those in the soil. Thus a lime-induced chlorosis may actually be an iron deficiency, because the high *p*H has made the soil iron unavailable to the plant. Sometimes the ratios of the elements in the root medium may be more important than the absolute quantities (e.g., the Fe:Mn and the Ca:B ratios). A recent method that overcomes the unavailability of iron due to high

*p*H is to apply *chelated* iron, i.e., iron combined with an organic compound in such a way that it cannot ionize and therefore cannot be precipitated (Stewart and Leonard).

Difficult as it sometimes has been to prove the essentiality of a nutrient element, it is often much more difficult to find out why it is essential, i.e., its role in the physiology of the plant. If the element is a constituent of a substance whose function in the plant is known, this, of course, explains why that element is essential. But it must always be remembered that a single element may play many different roles in the physiology of the plant. Nitrogen, for instance, is one of the elements in proteins; and life is therefore impossible without nitrogen. But it is also a constituent of phospholipids, vitamins, and chlorophyll, all of which play definite roles in the physiology of the plant. Nitrogen also occurs in many substances whose role in the plant is not known (e.g., plant bases). Phosphorus, like nitrogen, is a necessary constituent of many vitally important substances: nucleoproteins, phospholipids, enzyme components, etc. Sulphur occurs in proteins and some vitamins, as well as other substances such as glutathione. Magnesium is a constituent of chlorophyll, but this cannot be its only function, since it is also essential for nongreen plants, perhaps because it is an activator for many enzymes. Calcium occurs in the pectate that cements the walls of adjacent cells together; but unlike magnesium, it is not essential for some nongreen lower plants. However, its role is obviously much more fundamental than as a mere cementer of cells. There is reason to believe that protoplasm cannot exist in the absence of certain ions. In most organisms, Ca is undoubtedly one of these ions, K another. This, in fact, is almost the only concrete hypothesis of the role of K, even though it is needed in such large quantities by all plants. For unlike the above-mentioned substances, K is not a constituent of any known essential organic substance. It occurs practically solely in the ionic form. It may conceivably act as an enzyme activator, as does Mg, but its chief role seems to be to provide the necessary *ionic atmosphere* for protoplasm. The fact that it is needed in so much larger quantity than ions such as Ca and Mg may be related to the fact that a *balanced solu-*

tion for living cells consists of about 10 parts of K to 1 of Ca or Mg. It is not likely that negative ions such as SO_4 and PO_4 play a role in maintaining the normal ionic atmosphere of protoplasm, in view of the net negative charge on the proteins at the normal *p*H of protoplasm.

The role of the trace elements is in some cases partially understood; Fe and Cu are constituents of many enzymes (e.g., peroxidase, catalase, cytochrome oxidase in the case of Fe, tyrosinase and ascorbic acid oxidase in the case of Cu), Zn is a constituent of at least one enzyme (carbonic anhydrase), and Mn is an activator of several others. It is now known that Mo plays a role in nitrate assimilation. But in spite of the great amount of work on boron, its functions are completely unknown. According to the most recent theory, B forms a complex with sugars that penetrates into living cells more readily than the free sugars, and is therefore more readily translocated to the growing cells (Gauch and Dugger).

REFERENCES

1. Arnon, D. I. and P. R. Stout. The essentiality of certain elements in minute quantity for plants with special reference to copper. Plant Physiol., **14**:371–375. 1939.
2. Frey-Wyssling, A. Ernährung und Stoffwechsel der Pflanzen. Büchergilde Gutenberg, Zurich. 1945.
3. Gauch, Hugh G., and W. M. Dugger Jr. The role of boron in the translocation of sucrose. Plant Physiol., **28**:457–466. 1953.
4. Gericke, W. F. Soilless gardening. Prentice-Hall. 1940.
5. Hewitt, E. J. The role of the mineral elements in plant nutrition. Ann. Rev. Plant Physiol., **2**:25–52. 1951.
6. Hewitt, E. J. Sand and water culture methods used in the study of plant nutrition. Tec. Comm. No. 22, Commonwealth Agr. Bureau, East Malling. 1952.
7. Hoagland, D. R. Inorganic plant nutrition. Chron. Bot. 1944.
8. Hunger signs in crops. Amer. Soc. Agron. and National. Fert. Assn. Washington. 1941.
9. Miller, E. C. Plant physiology. McGraw-Hill. 1938.
10. Müller, D. Die physiologische Grundlage für die Mangelsymptome der Pflanzen. Physiol. Plant., **2**:11–23. 1949.

11. Robbins, W. Rei. Growing plants in sand cultures for experimental work. Soil Sci., **62**:3–22. 1946.

12. Robinson, W. O. and G. Edgington. Minor elements in plants and some accumulator plants. Soil Sci., **60**:15–28. 1945.

13. Stewart, Ivan and C. D. Leonard. Iron chlorosis—its possible causes and control. Citrus Magazine, June 1952.

14. Stiles, W. Trace elements in plants and animals. Macmillan. 1946.

15. Wallace, T. The diagnosis of mineral deficiencies in plants by visual symptoms. Chronica Botanica, 1949.

16. White, P. R. A handbook of plant tissue culture. Ronald. 1943.

Chapter 13

METABOLISM

A complete understanding of the true functions of the elements is possible only when the roles of all the substances found in the plant are known and when the formation and breakdown of these substances are fully understood. The chemical reactions resulting in the synthesis and breakdown of substances in the living and growing plant are known as plant *metabolism.* The synthesis of the organic substances from the raw materials (obtained from the soil and air) is usually called *anabolism;* the breakdown of organic substances to simpler organic or to inorganic substances is called *catabolism.* The anabolic are usually energy-absorbing and the catabolic energy-releasing reactions. A complete understanding of metabolism requires a consideration of the energy relations as well as of the chemical nature of the substances and the kinetics of the reactions.

Energy relations

Those chemical reactions that occur with a release of free energy (energy available to do work), so that the products have less free energy than the reactants, are known as *exergonic* reactions, those that involve an absorption of free energy are *endergonic* reactions. If only the heat of reaction is considered, the terms *endothermic* and *exothermic* are used. In some cases the heat of reaction and the free energy change are approximately the same, and the two sets of terms may be used interchangeably. But the free energy change is the more important quantity from the point of view of metabolism. Some reactions show practically no energy change and are called energetically neutral.

From the point of view of the second law of thermodynamics exergonic reactions are spontaneous. The endergonic, on the

other hand, cannot take place unless free energy is supplied from some external source. But it must be remembered that many reactions are taking place in the plant at any one time. Therefore, it is at least conceivable that the endergonic reactions may take place at the expense of the free energy released by the exergonic reactions. When this happens, endergonic reactions are called *driven* reactions; and since the two kinds are energetically linked, they are also called *coupled* reactions.

Thus the fixation of nitrogen by microorganisms

$$N_2 + 3\,H_2O \rightarrow 2\,NH_3 + \tfrac{3}{2}\,O_2$$

is an endergonic reaction which cannot go of itself because there is a free energy increase of 162 kcal. (Wohl and James). If, however, the oxygen produced can be used to oxidize glucose, the free energy released by this reaction is 172 kcal. The total (coupled) reaction is

$$N_2 + \tfrac{3}{2}\,H_2O + \tfrac{1}{4}\,C_6H_{12}O_6 \rightarrow 2\,NH_3 + \tfrac{3}{2}\,CO_2$$

This is accompanied by a free energy decrease of 10 kcal., and therefore is thermodynamically spontaneous.

Thus, in general, the anabolism of plants is possible only if catabolism is simultaneously taking place. This must always be remembered, though the two are studied separately for purposes of classification. It is, of course, true that the free energy needed to drive some endergonic reactions may be obtained from sources other than exergonic reactions. In the plant, this is true of photochemical reactions such as in the photosynthetic process, the free energy for which is obtained from sunlight.

Enzymes

As stated above, exergonic reactions are themodynamically spontaneous. This does not mean that the reactions will take place unaided, but simply that they are downhill reactions as far as energy is concerned. Substance X at energy level A can therefore be converted "spontaneously" to substances Y and Z at the lower energy level B (Fig. 30). The reason why X does not fall of itself to energy level B, at the same time splitting into Y and Z, is because there is an energy barrier. Substance X

is too stable to roll down to level B. It must first be *activated;* that is, it must be given some extra energy, for instance by heating, to enable it to go uphill and over the barrier.

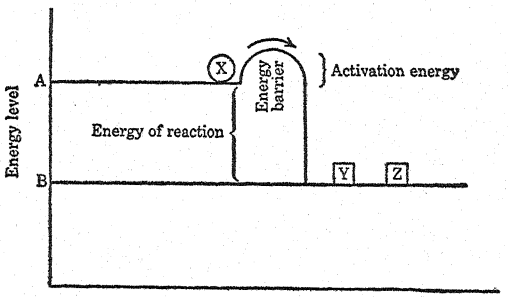


Fig. 30. Energy changes in a "spontaneous" (i.e., downhill) reaction, in which substance X is converted into substances Y and Z on falling from energy level A to energy level B.

But the reaction can be made to take place without heating in the presence of a highly reactive substance acting in extremely small quantities and known as a *catalyst.* If the reaction involves two substances, the catalyst may speed it up by adsorbing these substances, bringing them close enough together to reduce the energy barrier. Thus, when a stove is turned on, the gas does not burn until the activation energy is supplied by a match. Instead of a match, platinum black may be used. It acts as a catalyst by adsorbing the gases from the stove and the air, in this way starting the reaction without supplying heat.

The catalyst commonly forms an intermediate compound with the substance, converting it into a substance which is unstable. The energy barrier is therefore reduced to a small enough value for the energy of the molecules to overcome and the substance can fall to energy level B. When two substances react the steps will then be as follows:

$$a + \text{catalyst} \rightarrow \text{cat a}$$

$$\text{cat a} + b \rightarrow c + d + \text{catalyst} + \text{energy}$$

net reaction:

$$a + b \xrightarrow{\text{catalyst}} c + d + \text{energy}$$

The net result is that the catalyst has permitted the reaction to take place by removing or lowering the energy barrier (*energy of activation*). It is active in small quantities because it is regenerated after each reaction and able to combine with another molecule.

In the presence of such a catalyst, the exergonic reaction proceeds more or less rapidly with the release of free energy. The chemical substance or substances resulting from the first reaction may in turn be capable of reacting exergonically with another substance in the presence of a second catalyst, which is different from the first. Thus a whole chain of reactions may occur in a definite order, each link in the chain controlled by a specific catalyst. As a result, a complex substance may be broken down, step by step, to simpler and simpler substances, and at each step a packet of energy will be released, which may be used to drive an endergonic reaction.

By this gradual, orderly release of energy at the right time and in the right place, the plant is able to perform all its energy-consuming processes: protoplasmic streaming, active absorption of substances, cell growth, maintenance of gradients, etc. But none of these energy-consuming processes would be possible in the absence of the catalysts necessary for the energy-releasing reactions. And since there are many such reactions, there must also be many catalysts because they are highly specific. These organic catalysts that control the metabolism of living organisms are known as *enzymes*. Because of their high activity, their presence can be easily revealed. In many cases, it is necessary only to show that substance A will not break down of itself, but will when mixed with a plant juice. This indicates that the juice contains an enzyme that can catalyze the breakdown of A. For more rigid proof, this preliminary test is followed by other more exacting ones.

The number of known enzymes runs into the hundreds and is increasing daily. Seventy-two have been sufficiently purified and concentrated to obtain in the crystalline state (Schwimmer and Pardee). All such crystalline enzymes have proved to be proteins or protein complexes, usually with molecular weights from 36,000 to 260,000. Some of these appear to be enzy-

matically active without any nonprotein component. But most enzymes consist of two components, a protein and a nonprotein (Fig. 31). The nonprotein component may be firmly attached to the protein, when it is called a *prosthetic group*. Or it may be readily removed (e.g., by dialysis), in which case it is called a *coenzyme*. If the nonprotein component is a metal ion, it is sometimes called an *activator*, since the protein is inactive in

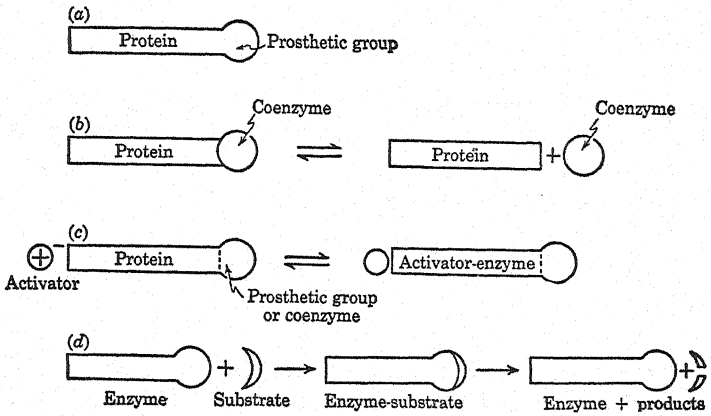
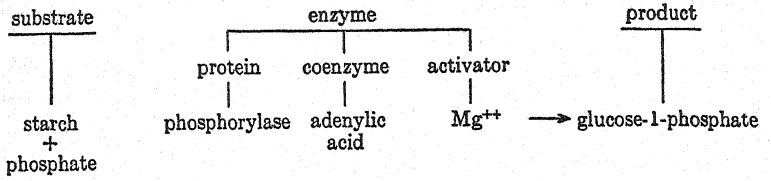


Fig. 31. Diagrammatic representation of enzyme systems: (a) protein plus nonremovable component; (b) protein plus removable component; (c) protein plus one or two removable components; (d) enzyme-substrate reaction.

the absence of the metal ion. (This explanation of activators may perhaps not apply in some cases.) The chemical substance that reacts in the presence of the enzyme to form some other substance is known as the *substrate*. In general it appears to be the prosthetic group (or coenzyme) that reacts with the substrate, leading to its conversion into a new substance or substrate, though it now appears that the sulfhydryl (SH) group of the protein may also react with the substrate (Barron). The protein part is thought to be responsible for the *specificity* of the enzyme. Thus several enzymes may contain the same prosthetic group, but because of the difference in the protein component, each will act specifically on one substance. For instance, tyrosinase and ascorbic acid oxidase both have Cu as a prosthetic group, yet the former acts on tyrosine (and several

similar substances) but not on ascorbic acid; the latter acts on ascorbic acid but not on tyrosine.

The following example illustrates some of the above terminology:



It is also an example of some of the inconsistencies in terminology and complexities of enzyme action. The phosphorylase is itself usually called an enzyme, though it is inactive in the absence of the other enzyme components. It would appear that this enzyme consists of three components. Other terminologies attempt to overcome these difficulties (e.g., *holoenzyme*, *apoenzyme*, and *coenzyme* for the complete enzyme, protein component, and nonprotein component, respectively). The very rapid action of enzymes may be illustrated by catalase, which controls the following reaction:

$$2\ H_2O_2 \xrightarrow{\text{catalase}} 2\ H_2O + O_2$$

One molecule of catalase can decompose 1000 molecules of H_2O_2 per second! The concentration of the enzyme affects the speed of the reaction, but not the position of equilibrium, and the final quantity of product is unchanged.

Due to the protein portion, enzymes are *heat labile;* that is, they are destroyed by 60°–100°C. The coenzyme component is *heat stable*, being unaffected by boiling. The enzyme is inactive at temperatures near freezing or in an acid *p*H in the case of some (e.g., trypsin). Low temperature inactivation is reversible, and so is *p*H inactivation in some cases, if the *p*H change is not too extreme. Poisons may inactivate many enzymes when present in very small quantities; for example, 0.0001 *M* cyanide inactivates many respiratory enzymes. These substances are frequently called *enzyme inhibitors*. Other inhibitors are sulfides, azides, fluorides, iodoacetates, etc. Some inhibitors are somewhat specific; that is, they inhibit only one

or a few enzymes. Frequently they inhibit by combining with the prosthetic group or with the sulfhydryl group of the protein, thus preventing it from combining with the substrate.

The terminology of enzymes is often arbitrary, though the tendency now is to name an enzyme after the substrate or the kind of reaction, adding the suffix -ase. Classification is based on the nature of the reaction rather than on the nature of the enzyme itself, since in so many cases the latter is unknown. It is therefore necessary to know something about the nature of the chemical reactions that take place in the plant. The majority fall into three main types:

1. *Hydrolytic and condensation reactions,* which may be represented as follows:

$$A - O - B + H_2O \underset{\text{condensation}}{\overset{\text{hydrolysis}}{\rightleftharpoons}} A - OH + B - OH$$

In most cases the hydrolysis results in a large release of energy, and these reactions are therefore usually practically irreversible. In other words, the enzyme usually speeds up the hydrolysis. If, however, the substance A − O − B is removed as rapidly as it is formed, the condensation can conceivably also be speeded up by the enzyme, at least in some cases.

2. *Transfer reactions,* which may be represented:

$$A - O - B + R - OH \rightleftharpoons A - OR + B - OH$$

These reactions usually result in such small energy changes that they are readily reversible. The enzyme can therefore speed up both the synthesis and breakdown reactions.

3. *Oxidation-reduction reactions,* which may be represented:

$$A \underset{\text{reduction}}{\overset{\text{oxidation}}{\rightleftharpoons}} A^+ + e^-$$

An example of this type is the oxidation of ferrous iron (Fe^{++}) to ferric iron (Fe^{+++}). Since electrons cannot be released in the free state in the plant, this reaction can occur only if there is another substance that accepts the electron. The complete reaction should therefore be represented:

$$\underset{\text{oxidized}}{A^+} + \underset{\text{reduced}}{B^-} \rightleftharpoons A + B$$

The production of ions is not necessary, for the electron may be transferred accompanied by a proton in the form of a hydrogen atom:

$$AH_2 + B \rightleftharpoons \underset{\text{oxidized}}{A} + \underset{\text{reduced}}{BH_2}$$

Or there may be an intramolecular transfer of electrons, e.g., to an atom such as oxygen, that has a marked tendency to accept two electrons:

$$CO + O \rightleftharpoons CO_2$$

In this case, the C atom is oxidized, the O atom reduced. In all cases, it must be realized that oxidation and reduction are inseparable; if something is oxidized, something else must be reduced.

Though these three types of reactions cover most of those occurring in the plant, some are not included. (For a more detailed and complete classification see Hoffmann-Ostenhof.)

Hydrolases or Hydrolytic Enzymes. Many complex organic substances in the plant are stable in the presence of H_2O, but are split hydrolytically if the necessary enzyme is present. They may be subdivided into four groups:

1. *Esterases.* The reaction is

$$R_1COOR_2 + H_2O \overset{\text{esterase}}{\rightleftharpoons} R_1COOH + R_2OH$$

where R_1 and R_2 are organic radicals that vary with the particular ester. Lipase controls hydrolysis of fats to fatty acids and glycerol. Other esterases are phosphatase, chlorophyllase, etc.

2. *Glycosidases or carbohydrases.* Invertase (sucrase) controls the hydrolytic splitting of sucrose to dextrose and fructose. Other carbohydrates or glycosides are hydrolyzed by amylase (diastase), cellulase, hemicellulase, maltase, emulsin, etc.

3. *Proteolytic enzymes.* Of these, the proteases split proteins to peptones, polypeptides, and amino acids. Papain and bromelin are the best-known plant proteases. Peptidases split peptides to amino acids.

4. *Amidases.* Urease controls the hydrolytic splitting of urea to CO_2 and NH_3. Asparaginase splits the amide asparagine.

Transferases. Many chemical groups (other than the H and OH of water) can be transferred by enzymes from one substance to another. Transmethylases transfer methyl groups, transaminases transfer amino groups, transphosphatases transfer phosphate groups, etc.

Oxidoreductases or Oxidation-Reduction Enzymes. These are the most heterogeneous of the three classes. The terminology of oxidation-reduction enzymes is complex and not uniform. In the case of reactions involving transfer of hydrogen atoms from one substance to another, the term dehydrogenation is commonly used, and the enzyme controlling the oxidation-reduction is called a *dehydrogenase;* for example,

$$\underset{\text{(ethyl alcohol)}}{CH_3CH_2OH} + \text{alcohol dehydrogenase} \rightarrow \underset{\text{(acetaldehyde)}}{CH_3CHO} + \text{reduced alcohol dehydrogenase}$$

In this reaction, the alcohol is oxidized (dehydrogenated) to acetaldehyde, and the dehydrogenase is reduced. The reduced dehydrogenase can in its turn be oxidized by another hydrogen acceptor and is then free to oxidize another molecule of ethyl alcohol. If the enzyme is reoxidized by molecular oxygen, it is called an *oxidase:*

$$(1) \qquad AH_2 + \text{enzyme} \rightarrow A + \text{enzyme } H_2$$

$$(2) \qquad \text{enzyme } H_2 + \tfrac{1}{2}\, O_2 \rightarrow \text{enzyme} + H_2O$$

One of the most important of these is cytochrome oxidase, another is tyrosinase. Two other well-known oxidation-reduction enzymes are peroxidase and catalase, enzymes that control the decomposition of H_2O_2. When peroxidase acts on peroxide, the oxygen released oxidizes another substance; when catalase acts on it, free molecular oxygen is released. The precise role of these two enzymes is not understood. Most of the oxidases contain metals in the prosthetic group: Fe in the case of cytochrome oxidase, peroxidase, catalase; and Cu in the case of tyrosinase.

In most of the above, the enzymes control catabolic reactions. However, anabolic reactions are also enzymatically controlled, though the enzymes have not been so well known until recently, partly because these energy-consuming reactions are not so easily brought about in the test tube. In some cases, the same enzyme may conceivably control the synthesis as well as the breakdown reaction, since the enzyme affects the kinetics of the reaction but not the equilibrium position. Thus hydrolysis of esters should be represented as an equilibrium reaction:

$$R_1COOR_2 + H_2O \overset{\text{esterase}}{\rightleftharpoons} R_1COOH + R_2OH$$

The esterase lipase has, indeed, been shown to induce the reverse condensation reaction, the synthesis of fat from fatty acid and alcohol.

But in at least some cases the synthesis proceeds along a totally different path from the breakdown. Thus sucrose is hydrolyzed in the presence of invertase:

$$\text{sucrose} + H_2O \overset{\text{invertase}}{\rightleftharpoons} \text{glucose} + \text{fructose}$$

The synthesis of sucrose is not simply the reverse of this reaction, but is

$$\text{glucose-1-phosphate} + \text{fructose} \underset{\text{phosphorylase}}{\overset{\text{sucrose}}{\rightleftharpoons}} \text{sucrose} + \text{phosphate}$$

and this synthesis is controlled by another enzyme. In fact, it can be produced in the test tube only in the absence of invertase, since the latter breaks down the sucrose as rapidly as it is formed.

Among the group of oxidation-reduction enzymes may also be included the *carboxylases* (Ochoa). Essentially all the CO_2 evolved in fermentation and respiration is produced by decarboxylation, and all the CO_2 absorbed in photosynthesis and allied processes is due to the reverse process of carboxylation. Since the two processes are at least theoretically reversible, it has become the custom to call all these enzymes carboxylases, regardless of whether they essentially control decarboxylation or carboxylation. Decarboxylation may be considered a dehydrogenation of the carboxyl:

$$R \cdot COOH \rightleftharpoons RH + CO_2$$

As a result, decarboxylation involves a large release of free energy and is a spontaneous reaction; carboxylation is possible only if free energy is provided from some outside source. Both processes are enzymatically controlled.

The enzyme pyruvic carboxylase can be used as a specific example. It is found in both higher and lower plants though not significantly in animals. It catalyzes the loss of CO_2 from pyruvic acid with formation of acetaldehyde, i.e., decarboxylation of pyruvic acid.

$$\underset{\text{pyruvic acid}}{CH_3COCOOH} \xrightarrow[\text{carboxylase}]{\text{pyruvic}} \underset{\text{acetaldehyde}}{CH_3CHO} + \underset{\text{carbon dioxide}}{CO_2}$$

Theoretically, it should be able to catalyze the reverse reaction as well, i.e., carboxylation of acetaldehyde to form pyruvic acid. But the decarboxylation involves so large a loss of free energy that the reaction is practically irreversible. The enzyme is a protein-diphosphothiamine-magnesium compound and the purest preparations obtained from yeast contain 1 mole diphosphothiamine and 1 gram-atom of Mg to 75,000 g. protein. Thus the thiamin (vitamin B_1) phosphate is the prosthetic group and Mg is the activator. The enzyme is able to decompose 700–900 moles of pyruvic acid per mole diphosphothiamine per minute. Heavy metals (Ag, Cu, Hg) strongly inhibit this enzyme. Though it is essentially a specific enzyme for pyruvic acid, nevertheless under certain conditions it can catalyze the decarboxylation of some other α-keto acids (acids with the CO or keto group in the α position, i.e., adjacent to the COOH group). So many *vitamins* have been shown to act as prosthetic groups of enzymes, that this may well prove to be their sole function.

One of the most amazing facts about enzymes is the tremendous number of different kinds that must coexist in the extremely small space occupied by the protoplasm of a single cell. The number is so large that it has even been suggested that all the cytoplasmic proteins are enzymes. Each must be able to control its own particular reaction without interference from—indeed, with the aid of—other enzymatically controlled reac-

tions. This orderly arrangement would not be possible if all the enzymes were mixed haphazardly in solution. It is not surprising, then, that so many of them have been found to be attached to particles in the cytoplasm, e.g., the mitochondria. If each one occupies its own place on such a particle, an orderly series of reactions can take place there.

REFERENCES

1. Barron, E. S. Guzman. Thiol groups of biological importance. Adv. in Enzym., **11**:201–266. 1951.
2. Bernfeld, P. Enzymes of starch degradation and synthesis. Adv. in Enzym., **12**:379–428. 1951.
3. Green, D. E. Mechanisms of biological oxidations. Cambridge University Press. 1940.
4. Hoffmann-Ostenhof, O. Suggestions for a more rational classification and nomenclature of enzymes. Adv. in Enzym., **14**:219–260. 1953.
5. Northrop, J. H., M. Kunitz, and R. M. Herriott. Crystalline enzymes. Columbia University Press. 1948.
6. Ochoa, Severo. Biological mechanisms of carboxylation and decarboxylation. Physiol. Rev., **31**:56–106. 1951.
7. Schwimmer, S. and A. B. Pardee. Principles and procedures in the isolation of enzymes. Adv. in Enzym., **14**:375–409. 1953.
8. Sumner, J. B. and K. Myrback. The enzymes. 4 vols. Academic. 1950–1952.
9. Wohl, K. and W. O. James. The energy changes associated with plant respiration. New Phyt., **41**:230–256. 1942.

Chapter 14

RESPIRATION

The most important catabolic, energy-releasing process in the plant is the oxidative breakdown of organic substances. Sugars are most commonly the starting point, and carbon dioxide and water are usually the end substances formed, though there are many exceptions. This process is known as *respiration.* Practically all the chemical reactions involved are enzymatically controlled. For some purposes, however, it is possible to ignore the enzymes and even the individual chemical reactions, and to consider the over-all process. Thus, if sugar is respired, respiration can be represented:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 674\ \text{kcal.}$$

This means that 180 g. (1 mole) glucose (or some other hexose) are oxidized by 192 g. (6 moles) of oxygen, releasing 264 g. (6 moles) of carbon dioxide, 108 g. (6 moles) of water, and 674 kcal. of heat. Thus each gram of glucose respired releases 3.74 kcal. of heat.

If this equation is correct, it should be possible to measure respiration by determining any one of the above five quantities. In practice, it is usually most convenient to determine the CO_2 released or the O_2 absorbed. If the correctness of the above equation is in doubt, it is necessary to measure both quantities. The molar ratio of CO_2 evolved/O_2 absorbed is then called the respiratory quotient. If this ratio is 1, the above equation would appear to be correct; that is, glucose or some other carbohydrate is apparently being respired to CO_2 and H_2O.

If substances other than glucose are respired, a different respiratory quotient usually results. Thus a fat would give

$$2\ C_{51}H_{98}O_6 + 145\ O_2 \rightarrow 102\ CO_2 + 98\ H_2O$$

The respiratory quotient is therefore 0.7. Similarly it can

readily be calculated for organic acids, e.g., 4 for oxalic acid ($C_2H_2O_4$) if it were respired. The value for proteins would be about 0.5. Actually, the quotient can vary from zero (no CO_2 evolved) to infinity (no O_2 absorbed). Some values that have been found experimentally are listed in Table 19.

TABLE 19: RESPIRATORY QUOTIENTS $\left(\frac{CO_2}{O_2}\right)$ IN DIFFERENT PLANTS

[After Thomas]

	Respiratory quotient
Leaves rich in carbohydrates	1
Darkened shoots of Opuntia (prickly pear)	0.03
Germinating starchy seeds	1
Germinating linseed (high fat)	0.64
Germinating buckwheat (high protein)	0.50
Germinating peas	1.5–2.4

In general, a respiratory quotient of less than 1 indicates that: (1) a substrate other than carbohydrate is being used; or (2) a carbohydrate is incompletely oxidized, for example, with the formation of organic acids that trap O_2 without releasing CO_2 and H_2O, as happens in succulents such as Opuntia in the above table; or (3) O_2 uptake occurs in processes other than respiration; or (4) the CO_2 evolved may be reassimilated.

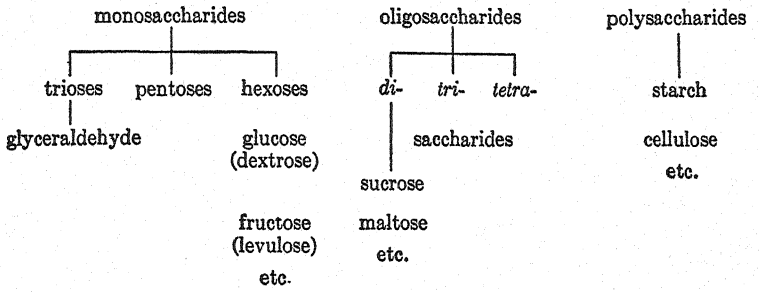
A respiratory quotient greater than 1 may mean that some high-oxygen substances, such as organic acids, are being respired, or that respiration is partially incomplete and therefore does not utilize molecular oxygen (see below).

In spite of these many exceptions, the respiratory quotient of higher plants is commonly 1. This means the equation given at the beginning of this section is probably correct for most higher plants under normal conditions, and carbohydrates are the main substrate for respiration. Many other lines of evidence confirm this conclusion. Even when fats are the main reserve material, they are first converted to sugars, which are then respired. Consequently, an understanding of respiration requires some knowledge of the carbohydrates.

Many different carbohydrates occur in the plant, some soluble, others insoluble. Each has an empirical formula consisting of C, H, and O, with the H and O in the proportions in

which they occur in water. But the properties of carbohydrates depend on their structural formulas. They consist of polyhydroxy (OH) aldehydes (substances with a CHO group) or ketones (substances with a CO group). In the case of the more complex carbohydrates, which are not themselves aldehydes or ketones, they give rise to polyhydroxy aldehydes and ketones (and to nothing else) when hydrolyzed.

They may be classified as follows:



Complete hydrolysis of starch, cellulose, and maltose yields only the monosaccharide glucose. Hydrolysis of sucrose yields both glucose and fructose. Due to their hydroxyl groups, carbohydrates form esters with acids. The most important of these in metabolism are the phosphate esters.

When leaves are starved (kept in the dark to prevent accumulation of photosynthate), the carbohydrates are used up in a few days (Fig. 32). These results indicate that some carbohydrates are respired more readily than others. Fructose is used up first, then sucrose and starch, and finally glucose. But the utilization of sucrose and starch is at first accompanied by an increase in glucose. Only after the other carbohydrates have been mainly exhausted does the glucose disappear. This indicates that the decrease in sucrose is initially mainly due to a respiratory breakdown of the fructose component.

The preferential respiratory breakdown of fructose in the starving barley leaves is in agreement with other results obtained with yeast. Whatever sugar (glucose, fructose, or mannose) is fermented by this organism, the diphosphoric ester of fructose is formed. Since this ester can be formed more readily

from fructose itself than from the other sugars, and since it is now known to be produced in higher plants during respiration (Albaum), it is not surprising that fructose is respired more

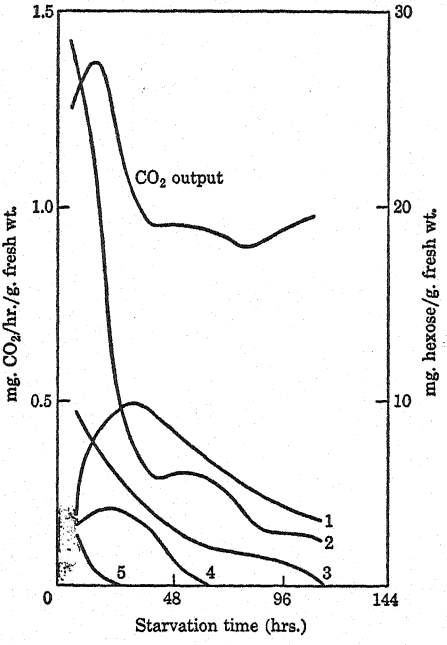


Fig. 32. Respiration of carbohydrates in barley leaves in the dark. Curve 1, glucose; 2, sucrose; 3, starch; 4, fructosan; 5, fructose. (After Yemm)

readily than the others. This formation of a phosphate ester of the sugar is the first step in respiration. It is called *phosphorylation.*

Anaerobic respiration

Many organisms are able to break down carbohydrates to CO_2 and some other substance (e.g., an alcohol or organic acid) without utilizing molecular oxygen. This process has been called by several names: *anaerobic respiration* because it may occur in the absence of air, *fermentation* because it is so often controlled by microorganisms or their enzymes (previously known as ferments), *glycolysis* ("glyco" means sugar) because

it is a breakdown of sugars. It is now known that all higher plants are capable of respiring anaerobically for varying lengths of time, but most die within a few days in the absence of molecular oxygen. Even when respiring aerobically in the normal manner, the first steps in the carbohydrate breakdown are almost completely identical with those in the anaerobic process. This is followed by a second series of steps involving utilization of molecular oxygen. Thus we can divide normal aerobic respiration into a first, anaerobic phase and a second, aerobic phase.

When higher plants respire in the absence of O_2, their respiration is usually partly or wholly alcoholic fermentation:

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 + 24\text{–}28\ \text{kcal.}$$

Much less energy is released in this way than in complete aerobic oxidation to CO_2 and H_2O. Substances other than ethyl alcohol may accumulate, e.g., glycerol, lactic acid, acetaldehyde, etc. In the case of some plants (e.g., potato tubers), acids accumulate but no alcohol. If only alcohol is produced, the ratio of alcohol to CO_2 is 1.04. The actual ratios found vary from this value all the way down to zero (Table 20).

Since so many plants accumulate ethyl alcohol under anaerobic conditions, early workers suggested that the normal aerobic respiration involves first the anaerobic breakdown to alcohol and CO_2, and second a further breakdown of the alcohol to CO_2 and H_2O. That the argument does not hold for some

TABLE 20: RATIOS OF CO_2 TO ALCOHOL IN PLANTS RESPIRING ANAEROBICALLY
[After Kostytchev]

Carrot roots	100:102
Oranges	100:70
Apples	100:42
Potato tubers	100:7

plants (e.g., potato) follows from the near absence of alcohol under anaerobic conditions. Furthermore, it was soon shown that higher plants do not oxidize alcohol very readily. It is now known that alcohol is not produced when the plant is respiring aerobically, though the first stage of aerobic respira-

tion is the same as alcoholic fermentation, up to a point previous to alcohol formation. Alcohol is thus an end product that occurs on a branch line instead of on the main line of reactions. This side branch operates only when respiration is anaerobic. The actual sequence in alcoholic fermentation is probably as follows:

hexose ⟶ hexosephosphates ⟶ triosephosphates ⟶

phosphoglyceric acid ⟶ phosphopyruvic acid ⟶

pyruvic acid ⟶ acetaldehyde ⟶ ethyl alcohol

When the respiration is aerobic, this stage goes only as far as pyruvic acid. Acetaldehyde and alcohol are on the anaerobic branch line.

Pyruvic acid is therefore the pivot. When aerobic respiration occurs, this substance is converted to another organic acid, which in its turn is changed to a third one, etc. (Fig. 33).

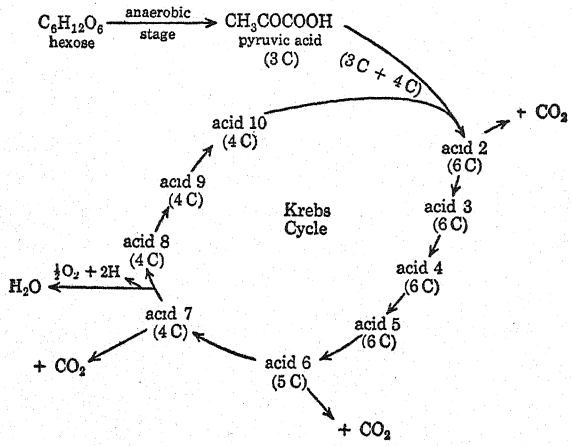


Fig. 33. Release of CO_2 as pyruvic acid is gradually broken down to CO_2 and H_2O (not all shown). The number of carbon atoms in each acid molecule is given in parentheses.

Each molecule of pyruvic acid is thus broken down in several steps to CO_2 and H_2O, by passing through a series of changes converting it successively into 6-, 5-, and 4-carbon acids. It forms the 6-carbon acid first by combining with a 4-carbon acid.

This cyclic series is known as the *Krebs* (or *citric acid*) *cycle*. Just which acids are involved in the higher plants is not known for certain, but the cycle probably includes citric (or its isomers), α-ketoglutaric, succinic, malic, fumaric, etc. It is possible that pyruvic acid is first converted to acetic acid before entering the cycle.

Both the anaerobic breakdown of sugars to pyruvic acid, and the aerobic breakdown of pyruvic acid in the Krebs cycle involve a release of respiratory energy. Just how this energy is utilized to drive endergonic processes is not completely understood. Recent evidence, however, indicates that it is primarily accomplished by a transfer of phosphate groups from the intermediates of respiration to the energy-consuming processes (Lipmann). In this transfer, the phosphate groups carry available energy with them and are therefore called *high-energy phosphates*. The process has been illustrated by analogy with a dynamo (the metabolic wheel) from which energy is brushed off and transported to the region of utilization by way of a substance known as adenylic acid (Fig. 34). When the phosphate gives up its energy, low-energy inorganic phosphate is regenerated, which may be again converted into the high-energy form by the metabolic wheel. The phosphates take part in the Krebs cycle as well as in glycolysis, though this is not shown in Fig. 33.

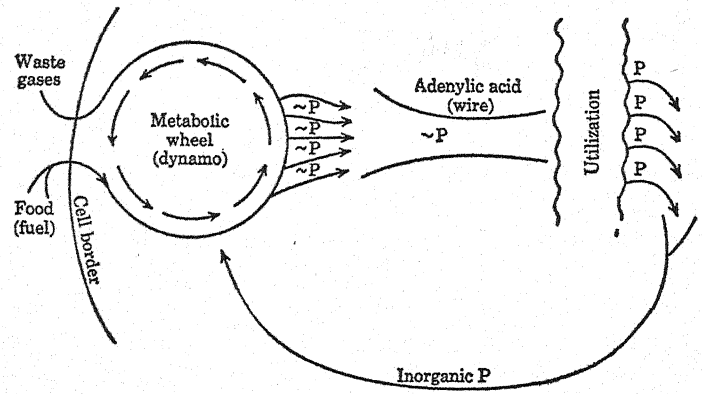


Fig. 34. Utilization of respiratory energy by means of high energy phosphates (~P). (After Lipmann)

All or nearly all the steps in both glycolysis and the Krebs cycle are, of course, controlled by enzymes. Many of these enzymes have been investigated, and most of those responsible for the Krebs cycle reactions have been found to occur in the mitochondria (Millerd).

Since respiration in higher plants is normally the energy-releasing breakdown of sugars to CO_2 and H_2O, this process obviously does not include all of catabolism. There must frequently also be a hydrolytic breakdown of more complex carbohydrates to sugars, of lipids to fatty acids etc., of proteins to amino acids. This process is sometimes known as *digestion*. The products of digestion may then be respired, or they may be resynthesized to other complex substances, or they may be translocated to other parts of the plant. Just as in the case of respiration, digestive processes are controlled by enzymes. In some cases, it is impossible to distinguish between digestion and respiration, for example, when starch is phosphorylated directly to hexose phosphate in glycolysis.

The rate of respiration is affected by many factors, both internal and external. From the equation, it is obvious that an ample supply of hexoses or other respirable material is essential for a rapid rate. The same is true of the O_2 supply to the respiring cells, at least if respiration is aerobic. Yet it has been shown that O_2 actually reduces the rate of breakdown as compared with anaerobic respiration. This is called the *Pasteur effect*. On the other hand, an accumulation of the end products reduces the rate. This fact is made use of in the storage of fruit. Air containing 10 per cent CO_2 retards respiratory breakdown, and therefore reduces sugar loss and prolongs the life of the fruit; but the oxygen content of the air must be maintained as high as normal to prevent anaerobic respiration.

The rate of respiration is, of course, markedly affected by temperature. A rise of 10°C usually increases the rate 2–3 times. This is the main basis for the low-temperature preservation of plant parts. Dropping the temperature from 30°C to 0°C may reduce the rate of respiration to 1/27, or $(\frac{1}{3})^3$, in this way keeping the reserves 27 times as long. Injury may markedly increase the respiration rate.

A striking variation in respiration rate may occur with age. Thus a developing fruit may show a marked drop during the early stages, followed by a rise, then another drop (Fig. 35). The first drop is perhaps more apparent than real, since the cell enlargement results in about as much of a decrease in per cent protoplasm (which is the only part of the cell capable of respiring) as the decrease in respiration per gram of total dry

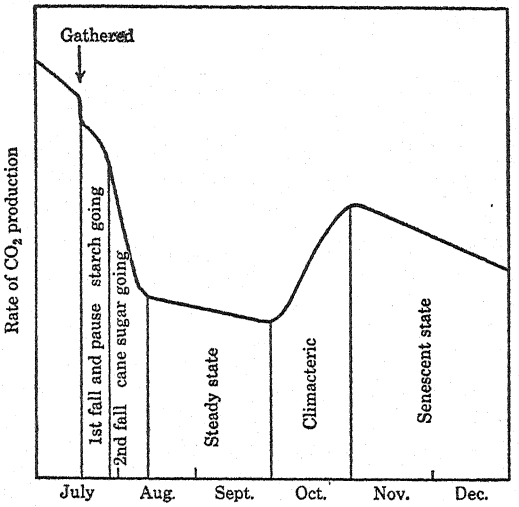


Fig. 35. Changes in respiration rate of apple fruit during growth and development. (After Kidd)

matter (Kidd and West). The rise and subsequent fall, however, are real changes, since they occur when the fruit is already full size. The *climacteric* rise accompanies ripening and is associated with the production of ethylene. Other volatile products, responsible for the flavor and aroma, also reach a maximum at this time, e.g., methyl, ethyl, and amyl esters of formic, acetic, caproic, and caprylic acids. Artificial ripening may be induced by treating the unripe fruit with ethylene gas (1:1000), and this is always accompanied by an increased rate of respiration. This climacteric period is followed by the final period of *senescence*, during which the respiration process seems to be thrown out of gear. Not only does the total rate drop

rapidly, but ethyl alcohol and acetaldehyde steadily accumulate, the latter giving the off taste. This seems to indicate that oxidative processes are hindered and that anaerobic respiration becomes more pronounced. Other metabolic changes also occur; for example, insoluble pectins that normally cement cells together are converted to soluble pectin by the enzyme protopectinase. As a result the cells separate and the flesh becomes mealy. In the case of fruit such as tomato and grapes, even the cell walls dissolve and the free protoplasts are relased. These later break down. This whole self-digestive process is known as *autolysis*.

REFERENCES

1. Albaum, H. G. The metabolism of phosphorylated compounds in plants. Ann. Rev. Pl. Physiol., **3**:35–58. 1952.
2. James, W. O. The respiration of plants. Ann. Rev. Biochem., **15**:417–434. 1946.
3. Kidd, F. L. Respiration of fruits. Nature, **135**:327–330. 1935.
4. Kidd, F. L. and C. West. Respiratory activity and duration of life of apples gathered at different stages of development and subsequently maintained at a constant temperature. Plant Physiol., **20**:467–504. 1945.
5. Kostytchev, S. Chemical plant physiology. Trans. and ed. by C. S. Lyon. Blakiston. 1931.
6. Lipmann, F. Metabolic generation and utilization of phosphate bond energy in *Advances in Enzymology*, Vol. I, F. F. Nord and C. H. Werkman, eds., Interscience. 1941.
7. Millerd, A. Respiratory oxidation of pyruvate by plant mitochondria. Arch. Biochem. and Biophys., **42**:149–163. 1953.
8. Thomas, M. Plant physiology. London. J. and A. Churchill. 1949.
9. Yemm, E. W. The respiration of barley plants. II. Carbohydrate concentration and carbon dioxide production in starving leaves. Proc. Roy. Soc. Lond., **117 B**:504–525. 1935.

Chapter 15

PHOTOSYNTHESIS

The anabolic counterpart of respiration is *carbon assimilation.* When this process is dependent on light energy, it is called *photosynthesis.* In many respects it is the direct opposite of respiration, and can actually be represented crudely by the same equation turned around:

$$6\ CO_2 + 6\ H_2O + \text{light energy} \xrightarrow{\text{chlorophyll}} C_6H_{12}O_6 + 6\ O_2$$

Instead of sugar being broken down into CO_2 and H_2O, the latter are built up to sugar; instead of O_2 being absorbed, it is released; instead of energy being released, it is absorbed in the form of light energy and converted into the chemical energy of the sugar molecule; instead of oxidation of a carbon-containing compound, CO_2 is reduced to hexose, and H_2O is oxidized to O_2.

Just as in the case of respiration, the rate of photosynthesis can be theoretically measured by determining any one of the quantities in the above equation; but the most easily determined are the CO_2 absorbed and the O_2 released. The photosynthetic quotient is O_2/CO_2, and as in the case of the converse respiratory quotient CO_2/O_2, is usually equal to 1. However the photosynthetic quotient has not proved to be as valuable a ratio as the respiratory quotient, partly because photosynthesis, unlike respiration, goes all the way or not at all (as far as gross measurements of O_2 and CO_2 are concerned), partly because carbohydrates are nearly always the end point.

Just as in the case of respiration, the above equation for photosynthesis is simply a statement of the relative quantities of raw materials and final products. It reveals nothing about the individual chemical reactions that follow each other, step by step, in an orderly and integrated fashion. Up to about 10 years ago, nothing was known about these individual chemical reactions, though several theories, now completely dis-

credited, had been proposed. During recent years, however, great progress has been made, and we now know several of the intermediate substances, and can at least theorize reasonably as to the probable steps, though they are still not so well known as in the case of respiration.

This great progress in our understanding of the photosynthetic intermediates is mainly due to the use of isotopes. The important contributions of these elements to our understanding of permeability and translocation have already been mentioned. In the case of photosynthesis, they have been responsible for opening a whole new field of investigations. The first isotope used was heavy oxygen, O^{18}. Only when heavy oxygen was incorporated in the H_2O supplied to the plant, was it recoverable (in the same concentration) in the oxygen released. Though this evidence is not as conclusive as it at first appears (Brown and Frenkel), it agrees with other evidence indicating that the source of the O_2 evolved in photosynthesis is the H_2O and not the CO_2. Consequently, the equation as given above is not correct and must be modified as follows:

$$6\ CO_2 + 12\ H_2O^* + \text{light energy} \rightarrow C_6H_{12}O_6 + 6\ H_2O + 6\ O_2^*$$

The asterisk indicates the same atoms of oxygen. To balance the equation, twice as many water molecules must be involved as in the first over-all equation. Even this equation is not necessarily correct. It simply shows the *minimum* number of water molecules. As far as the above evidence is concerned, there may be 18 or 24, or some other multiple, on the left side, and 12 or 18 etc., respectively, on the right side of the equation.

Even more important results have been obtained with the use of carbon isotopes. The normal isotope of carbon is C^{12}, heavy carbon (the stable isotope) is C^{13}, and there are two radioactive isotopes, C^{11} and C^{14}. Only the radioactive isotopes have been used in studies of photosynthesis, and although the short-lived C^{11} was used at first, all the more recent work has been with the longer-lived C^{14}. The plant is supplied with $C^{14}O_2$ in the light for a few seconds, or minutes, then quickly killed and analyzed. Those substances that contain C^{14} must have been synthesized by the plant from the $C^{14}O_2$. If the plant is

allowed to use the $C^{14}O_2$ for different lengths of time, the substance formed in the shortest period is obviously the first in the chain of reactions. In this way it was proved that a phosphoric ester of a 3-carbon compound, phosphoglycerate, is the first or almost the first substance formed in photosynthesis, appearing within 2–5 seconds. Other intermediates such as organic acids and hexose-phosphates are formed later in the process. The following are some of the suggested steps in the sequence of reactions (Benson and Calvin): carbon dioxide + glycolic acid → phosphoglycerate → triose phosphate → hexose phosphates → sugar or starch. But it is still not possible to state with certainty what the actual chemical reactions are, or what enzymes are involved, though recent work has yielded evidence of the possible roles of one or two enzymes.

Just as in the case of respiration, photosynthesis includes not only chemical changes, but energy changes as well. These energy changes cannot be studied by use of C^{14}; for the synthesis of carbon compounds is not itself a photosynthetic process and does not require light. Animal cells and colorless plant cells, or green plant cells in the dark, are all able to build up more or less complex carbon compounds from CO_2 in the dark. This process is known as *dark carbon assimilation.* The energy for the process is obtained from the simultaneous respiratory breakdown of sugars. When carbon assimilation occurs in the light in green plants, the energy is obtained from the absorbed light. Dark carbon assimilation is of course wasteful, since it drains the reserves; photosynthesis, on the other hand, increases the reserves.

In order to utilize the light energy, there must be a photochemical reaction or series of reactions in the process of photosynthesis. Although these photochemical reactions play no direct part in the actual steps of carbon assimilation described above, they are just as essential to the process, since they provide the energy. As in the case of the carbon assimilation which occurs in the dark, the photochemical reactions have been intensively investigated in recent years, and much new information has accumulated in the past decade. The most important tool in these investigations has been the so-called *Hill reaction.*

This is the name given to the photosynthetic release of molecular oxygen by free chloroplasts in the presence of an oxidizing substance (such as ferric salts, quinones, etc.). The Hill reaction takes place without any carbon assimilation, and therefore permits a separate study of the photochemical phase of photosynthesis. The free chloroplasts are obtained by grinding green leaves and centrifuging successively at increasing speeds to remove sediment, and to precipitate the chloroplasts. The latter are then resuspended in solutions of various kinds and exposed to light under conditions permitting measurement of O_2 evolution.

In this way, it has been found possible to obtain nearly as rapid evolution of O_2 from isolated chloroplasts as from the normal leaf, though no carbon assimilation occurs. But it must be emphasized that the chloroplast is not a complete system in itself even for O_2 evolution. It must be supplied with an oxidizing agent which it can reduce in the light. That the evolution of O_2 is controlled by enzymes has been shown by the use of inhibitors such as urethane, which also inhibit photosynthesis in the normal leaf. On the other hand, cyanide in low concentrations does not inhibit the Hill reaction, though it does inhibit photosynthesis in the leaf, showing that its effect is on the carbon assimilation and not on the photochemical evolution of O_2. By supplying the necessary enzymes and substrates not present in the chloroplasts, it is now even possible to obtain CO_2 assimilation with free chloroplasts in the light (Vishniac and Ochoa).

By use of the Hill reaction and other methods, attempts are constantly being made to find out more about the photochemical phase of photosynthesis. By analogy with respiration, it is possible that the absorbed light energy is transformed into chemical energy in the form of high-energy phosphates. The process of carbon assimilation is presumably driven by this chemical phosphate energy. That is perhaps why the first substance identified in carbon assimilation is phosphoglycerate.

Some aspects of the role of chlorophyll in the process of photosynthesis are clear. No photochemical process can take place unless light energy is absorbed. It is the chlorophyll that

absorbs the light energy, which it then transfers in some unknown manner (perhaps by means of high energy phosphates) to the CO_2 or some intermediate of carbon assimilation.

There are two chlorophylls, one bluish green (chlorophyll a), the other yellowish green (chlorophyll b). Both occur only in the chloroplasts (in higher plants) usually in the ratio of 3a:1b. With them are two yellow pigments, carotin and xanthophyll, in about the same molar quantity as chlorophyll b. The four are known as the *chloroplast pigments*. The chlorophyll molecule is complex. It contains Mg, as well as C, H, N, and O. It cannot be synthesized in the absence of Fe. One explanation is the suggestion that the finished molecule results from a replacement of Fe in the molecule by Mg. The portion of the molecule that contains Mg is chemically related to the heme of the blood.

That chlorophyll is the energy absorbing substance can be shown in several ways. The *absorption spectrum* of chlorophyll

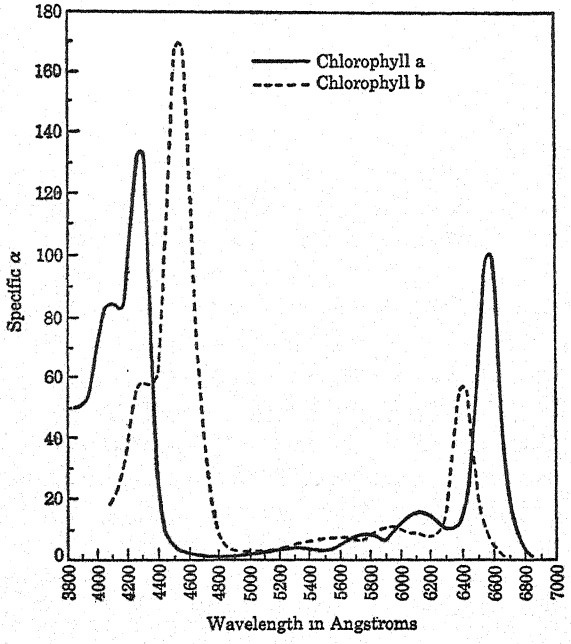


Fig. 36. Absorption spectra of chlorophylls a and b in ether. (After Zscheile and Comar)

(Fig. 36) is the same as the *action spectrum* of photosynthesis; that is, chlorophyll absorbs most intensely in the red, at the wavelength that permits the most rapid photosynthesis; it absorbs least in the green, and this light (at the same intensity as the other wavelengths) permits only the slowest rate of photosynthesis. It is interesting that all wavelengths of light do permit photosynthesis, though in view of the above absorption characteristics of chlorophyll, red is the most efficient. Recent evidence (Rabinowitsch) indicates that the yellow plastid pigments may also serve to absorb light that may be used photosynthetically.

TABLE 21: RATE OF PHOTOSYNTHESIS IN SOME PLANTS AT TEMPERATURES OF 18–20°C., AT MAXIMUM OR NEAR MAXIMUM LIGHT INTENSITIES, AND WITH NORMAL CO_2 CONTENT OF THE AIR (0.03%, or 0.56 mg. per l)
[After Lundegårdh]

Plant	*mg.* CO_2 *per 50 cm.² leaf surface per hr.*	*Carbohydrate* ($C_6H_{12}O_6$) *synthesized in g. per m² leaf surface per hr.*
Potato	9.57	1.30
Tomato	8.42	1.15
Sugar beet	9.26	1.26
Spinach	9.78	1.33
Vicia Faba	8.83	1.20
Phaseolus vulgaris	9.27	1.26

The rate of photosynthesis (Table 21) is controlled by many factors, both internal and external. When several factors are investigated, it is usually found that only one limits the rate of photosynthesis; that is, this one factor when increased, will markedly increase the rate of photosynthesis, whereas the others when increased have little effect on the rate of the process (Fig. 37). This fact has been called the *law of limiting factors*. Thus, though light intensity, temperature, and CO_2 concentration are all important factors, if the temperature on a normal day is around 0°C., no increase in light intensity or CO_2 concentration will affect the rate of the process, but an increase in temperature will produce a corresponding increase in rate of photosynthesis (Fig. 38), provided that the other factors are not limiting.

As in the case of respiration, this effect of temperature is pro-

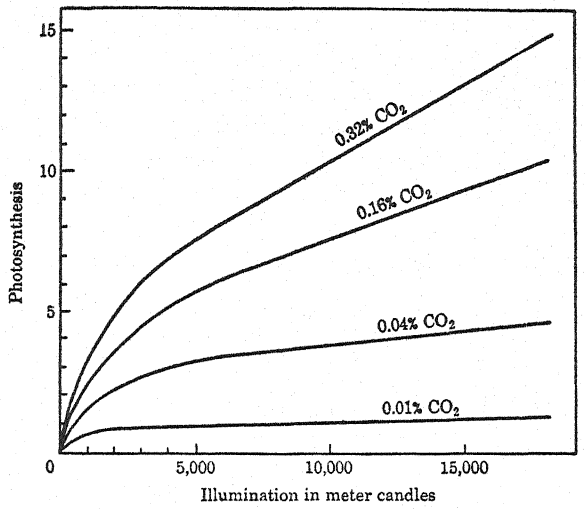


Fig. 37. Photosynthesis of an aquatic plant (Fontinalis) at different light intensities and CO_2 concentrations. (After Harder)

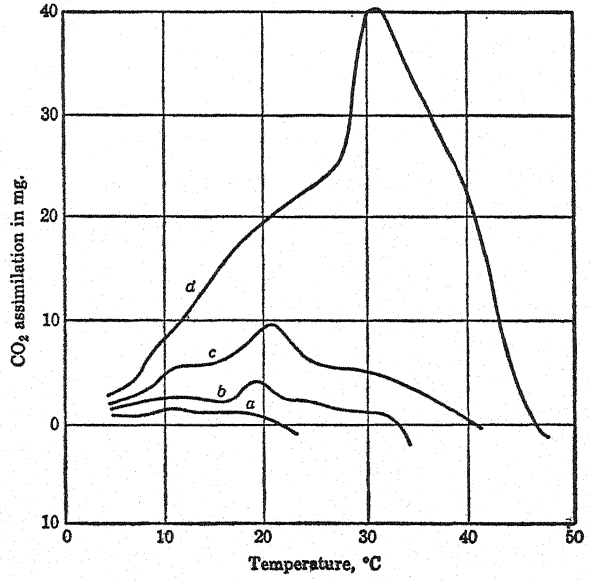


Fig. 38. Effects of CO_2 concentration, light, and temperature on photosynthesis by potato leaves. Curve *a*, very weak light and very low CO_2 concentration; *b*, 1/25 normal light and 0.03% CO_2; *c*, normal light and 0.03% CO_2; *d*, normal light and 1.22% CO_2. (After Lundegårdh)

nounced. A rise of 10°C. may increase the rate of photosynthesis 2–3 times or sometimes even more. This is typical of ordinary chemical processes. Since physical processes are not so markedly affected by temperature, photochemical reactions show little change; for example, a 10°C. rise in temperature usually increases the rate of a photochemical reaction by only about one-tenth. This fact was long taken as evidence that the dark chemical reactions of photosynthesis are slower than the photochemical reactions; that is, the former are limiting. Indeed, the marked temperature effect was the earliest evidence of the fact that not all of the photosynthetic process requires light. In memory of the man who first observed this, the term *Blackman reaction* was used for the dark chemical reactions. Another early proof of the existence of dark chemical reactions was the fact that intermittent light can permit just as rapid a rate of photosynthesis as continuous light, if the light and dark periods are short enough, even though the dark periods between flashes reduce the total light received to about half as much per hour. However, if the alternating light and dark periods are increased to about 1 minute each, practically no photosynthesis takes place in some plants. This is due to the so-called *induction period:* after exposure to darkness for a minute or more, it takes the plant a full minute to reach its normal rate of photosynthesis in the light.

The efficiency of photosynthesis is not high under normal conditions. Only about 2 per cent of the incident light energy is ordinarily used in photosynthesis, or 3 per cent of the absorbed light energy. In weak light and with an ample supply of CO_2 the efficiency may rise to 30 per cent.

Whether or not respiration continues at the normal rate during photosynthesis is difficult to determine. If it does, the measured CO_2 absorption (or O_2 evolution) is really due to the excess of photosynthesis over respiration and is therefore a measurement of the *net rate of photosynthesis.* To get the true rate of photosynthesis it would be necessary to add to the CO_2 absorption the CO_2 evolution (i.e., respiration) of the same plant when kept in the dark. Some recent workers have, however, suggested that respiration does not occur at all when a

plant photosynthesizes, and that the latter process takes over the functions of respiration. Only recently has this problem been tackled directly (Van Norman and Brown). By supplying the plant with atmospheric oxygen containing heavy oxygen (O^{18}) and water with the normal isotope (O^{16}), it was possible to measure respiration directly while photosynthesis was taking place. In the case of both barley and the green alga Chlorella, light failed to inhibit respiration.

The total gain in carbohydrate by a plant over a 24-hour period depends not only on the measured rate of photosynthesis during daylight, but also on the night respiration, since this uses up some of the accumulated carbohydrate. Thus the yield of a crop may sometimes be markedly increased by lowering the night temperature, since this will increase the net assimilate per 24-hour period.

It should be pointed out that besides the ordinary green plants, there are bacteria that photosynthesize. They also possess chlorophyll and assimilate carbon compounds at the expense of CO_2 in the light, but they do not evolve O_2. In fact, they are able to photosynthesize only under anaerobic conditions.

REFERENCES

1. Benson, A. A. and M. Calvin. The path of carbon in photosynthesis. VII. Respiration and photosynthesis. J. Exp. Bot., 1:63–68. 1950.
2. Brown, A. H. and A. W. Frenkel. Photosynthesis. Ann. Rev. Plant Physiol., 4:23–58. 1953.
3. Franck, J. and W. E. Loomis. Photosynthesis in plants. Mon. Am. Soc. Plant Physiol., 1949.
4. Gaffron H., and E. W. Fager. The kinetics and chemistry of photosynthesis. Ann. Rev. Plant Physiol., 2:87–114. 1951.
5. Harder, R. Kritische Versuche zu Blackmans Theorie der "begrenzenden Faktoren" bei der Kohlensäure-assimilation. Jahr. f. wiss. Bot., 60:531–571. 1921.
6. Lundegårdh, H. Klima und Boden. Gustav Fischer. Jena. 1949.
7. Rabinowitch, E. I. Photosynthesis and related processes. Interscience. 1945.

8. Rabinowitsch, E. I. Photosynthesis. Ann. Rev. Plant Physiol., **3**:229–265. 1952.

9. Van Norman, R. W. and A. H. Brown. The relative rates of photosynthetic assimilation of isotopic forms of carbon dioxide. Plant Physiol., **27**:691–709. 1952.

10. Vishniac, Wolf, and Severo Ochoa. Fixation of carbon dioxide coupled to photochemical reduction of pyridine nucleotides by chloroplast preparations. J. Biol. Chem., **195**:75–93. 1952.

11. Zscheile, F. P. and C. L. Comar. Influence of preparative procedure on the purity of chlorophyll components as shown by absorption spectra. Bot. Gaz. **102**:463–481. 1941.

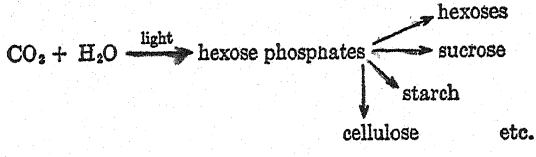
Chapter 16

OTHER ASPECTS OF METABOLISM

The process of photosynthesis is responsible for the formation of sugars and starch in green plants, respiration (and dark carbon assimilation) for the formation of organic acids and some related substances. But there are many other substances synthesized by the plant. These may be roughly classified as follows:

1. Other carbohydrates
2. Nitrogen-containing substances
3. Lipids
4. Miscellaneous (lignins, glycosides, terpenes, etc.)

1. Some of the other carbohydrates are closely related to the products of photosynthesis and may be formed from the latter (perhaps from the hexose phosphates) in the presence of the necessary enzymes. The following relationships seem logical; though not proved experimentally:



However, the mode of origin of most of the carbohydrates is not fully understood.

2. There are many nitrogen-containing substances in the plant, of which the proteins are, of course, of prime importance. When a high-protein seed germinates, there is a rapid disappearance of protein. Usually one or both of the amides, asparagine and glutamine, account for half or more of the lost protein. But along with the amides, some dozen or more amino acids have been identified. The exact number that arise is not known because of the small quantities in which they usually

accumulate. To a large extent they are quickly converted to something else, e.g., the amides mentioned above. If the proteins are hydrolyzed artificially, some 15–20 or more different amino acids arise, even in the case of the simplest proteins.

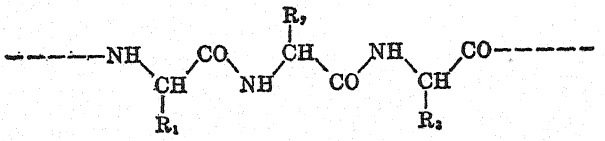
An understanding of protein breakdown and synthesis therefore depends on a knowledge of amino acid structure and synthesis. All the amino acids found in proteins are of the α-amino type (i.e., the amino group is on the so-called α-carbon atom, which is next to the carboxyl group):

$$\begin{array}{lll} \mathrm{R\,CH\cdot COOH} & \text{such as} & \mathrm{CH_2NH_2COOH}\ \text{glycine} \\ \mathrm{NH_2} & & \mathrm{CH_3CHNH_2COOH}\ \text{alanine, etc.} \end{array}$$

Some amino acids have more than one carboxyl group and are therefore acidic, others have more than one amino group and are basic. Those with one of each group are neutral. In the formation of proteins, the amino acids link together as follows:

$$\mathrm{NH_2\overset{R_1}{\overset{|}{C}}HCOOH + NH_2\overset{R_2}{\overset{|}{C}}HCOOOH \longrightarrow NH_2\overset{R_1}{\overset{|}{C}}H{-}CO{-}NH{-}\overset{R_2}{\overset{|}{C}}HCOOH + H_2O}$$

The protein chain therefore has the following make-up:



Thus the plant must first synthesize amino acids before it can make any proteins. One characteristic of the higher plant is this ability to synthesize from inorganic nitrogen all the 20-odd amino acids necessary for the formation of its proteins. Animals, on the other hand, must be supplied with the ten *essential amino acids*, that is, the ten amino acids that they are unable to synthesize. The other dozen or so they can synthesize from these 10.

When NO_3^- is used, the plant must first convert it to the NH_3 form before synthesizing amino acids. This would seem to indicate that the NH_4^+ form would be a more efficient source of N, since respiratory energy must be used to reduce NO_3^- to this form. However, under suitable conditions both forms are

equally satisfactory sources of N, and the NO_3^- form is suitable over a larger range of conditions (e.g., over a wider *p*H range).

When the NO_3^- is reduced to NH_3, the evidence indicates that it quickly combines with organic acids (probably those of the Krebs cycle) to form amino acids. Specific enzymes are required for these syntheses (aminases, transaminases, etc.).

In many cases the amino acid synthesis occurs in the roots of a plant (e.g., the apple) and the amino acids are translocated to the growing shoots, where they are built up to form proteins. In other plants (e.g., wheat), however, the inorganic N is translocated directly to the leaves and synthesized there to amino acids and proteins. The synthesis in the roots occurs, of course, in the dark. The synthesis in the leaves, on the other hand, may occur only in the light, at least in the case of certain plants. The tracer work on photosynthesis has, in fact, shown that some amino acids are synthesized within about five minutes after photosynthesis has started. There is therefore apparently a close connection between photosynthesis and amino acid synthesis in green cells.

It should be pointed out that although the gross amino acid composition of some proteins has been reasonably well worked out, there is not a single protein whose actual structure (the spacial arrangement of the amino acids) is known. Some of the proteins are so-called *conjugated proteins*. They consist of a protein molecule linked to nonprotein molecules (lipids, carbohydrates, nucleic acids, etc.). Many other nitrogen-containing substances are found in the plant (amines, betaines, alkaloids, purines). Many of these appear to be formed from the amino acids.

3. Lipids. Among the fatty substances or lipids, the ones that have been investigated the most are the fats (glyceryl esters of organic acids) and waxes (alcohol esters of organic acids). Little is known of their metabolism. Their synthesis occurs at the expense of sugars, and their hydrolysis is followed by the appearance of sugars, but how these conversions occur is not known. Thus ripening nuts show an increase in fats and a decrease in carbohydrates. A germinating fatty seed shows the opposite change.

4. Glycosides are commonly found in plants. They are formed by combination of sugars with a large number of different organic substances found in the plant. The blue and red pigments (anthocyanins), and some yellow pigments (flavones) are glycosides, as are the tannins. The metabolism of these and most other substances found in the plant is little understood.

There are innumerable other substances in the plant. In most cases their metabolism is completely unknown.

REFERENCES

1. Benson, A. A. and M. Calvin. The path of carbon in photosynthesis. VII. Respiration and photosynthesis. J. Exp. Bot., 1:63–68. 1950.
2. Bonner, J. Plant Biochemistry. Academic. 1950.
3. Chibnall, A. C. Protein metabolism in the plant. Yale University Press. 1939.
4. Gortner, R. A. Outlines of Biochemistry. Wiley. 1949.

Part IV

GROWTH AND DEVELOPMENT

Chapter 17

GROWTH

Of all the phases of physiology, growth and development are the most complex, since they depend on all the other physiological processes. It is impossible to understand plant growth without a knowledge of the basic facts of cell physiology, transfer of materials, mineral nutrition, and metabolism. Besides these processes, other factors specific to growth and development must be introduced. Foremost among these are the growth substances: hormones, or auxins. The advances made in this field during the past three decades are unsurpassed by those in any other phase of plant physiology. Yet it must be remembered that these substances are essentially switch mechanisms for turning growth or development on or off. No understanding of the switch can substitute for an understanding of the growth mechanism itself.

The first requirement for an understanding of growth is a suitable method of measuring it. When a plant grows, it increases in size. The ideal method of measuring growth would therefore be to determine the volume of the plant or plant part. But this is usually difficult to do with any degree of accuracy. In the case of growing leaves, the area is more readily determined; in the case of stems and roots, a simpler measurement is length. The easiest and most accurate measurement, however, is usually the weight of the plant or plant part. But this leads to some complicating factors. It must be decided, for instance, whether fresh or dry weight is a more nearly correct measurement of growth. In the case of germinating seeds, growth occurs rapidly, yet dry weight decreases steadily for some time because of rapid loss of reserves. During this period, only the fresh weight reveals the amount of growth. The same is true in the case of a growing tree seedling in the spring. On the other hand, during midsummer when the tree has stopped

growing, there is a steady increase in dry weight due to an accumulation of reserves. Dry weight can therefore serve as a measurement of growth only when it does not include any significant changes (increases or decreases) in the plant's food reserves. It is a satisfactory measurement of growth of seedlings only if the endosperm or seed leaves are not included.

The cellular basis of growth

The mere increase in size of a plant or a plant part is not necessarily the result of growth. The first uptake of water by dry seeds is purely an imbibition process and may occur equally in living and dead seeds. Volume or fresh weight measurements in this case would reveal a spurious growth, and increased volume is therefore not an adequate definition of growth. Obviously, then, growth must be defined in another way, preferably on a cellular basis. Since the growth of a plant occurs in regions known as meristems, the cellular changes in these tissues should throw some light on the process. Three main changes occur in meristems: *cell division*, *cell enlargement*, and *cell differentiation*. That growth of a plant part may be due to either cell division or cell enlargement is shown in Table 22. In this case both P and N increased root growth, P due to increased cell division, N due mainly to increased cell enlargement.

TABLE 22: GROWTH OF ISOLATED WHEAT ROOTS WITH LOW AND HIGH PHOSPHATE AND NITRATE [After Burström]

Nutrient solution	*Increase in root length (mm.)*	*Cell length (μ)*	*Increase in cell number (longitudinally)*
Low P, low N	13.7 ± 1.7	177 ± 3	35 ± 4
High P, low N	25.5 ± 2.0	170 ± 3	120 ± 5
Low P, high N	25.2 ± 1.3	269 ± 4	51 ± 3

But it must be realized that even growth due to cell division is possible only because the daughter cells enlarge to the size of the mother cell before they again divide. Consequently, the growth of a plant or plant part is essentially an increase in size due to cell enlargement, provided that this cell enlargement is of the so-called plastic or irreversible type, not simply an elastic or reversible increase due to increased cell turgor.

According to the classical concept of cell growth, the motivating force is turgor pressure. Yet it is a fact that mature,

TABLE 23: WALL PRESSURES OF DEVELOPING CELLS IN THE HYPOCOTYL OF HELIANTHUS SEEDLINGS. MAXIMUM RATE OF CELL ENLARGEMENT IN ZONE 2. BEYOND THIS, INCREASING ZONE NUMBER CORRESPONDS TO INCREASING MATURITY [After Beck and Andrus]

Zone	*Distance from cotyledons (mm.)*	*Wall pressure (atm.)*	
		epidermis	*cortical cells*
1	0–5	2.0	0.235
2	5–10	1.44	0.210
3	10–15	1.39	0.120
4	15–20	1.14	0.120
5	20–25	2.05	0.42
6	25–30	2.28	...
7	30–35	2.58	0.42

nongrowing cells usually have higher turgor (and wall) pressures than growing cells (Table 23). There is obviously another

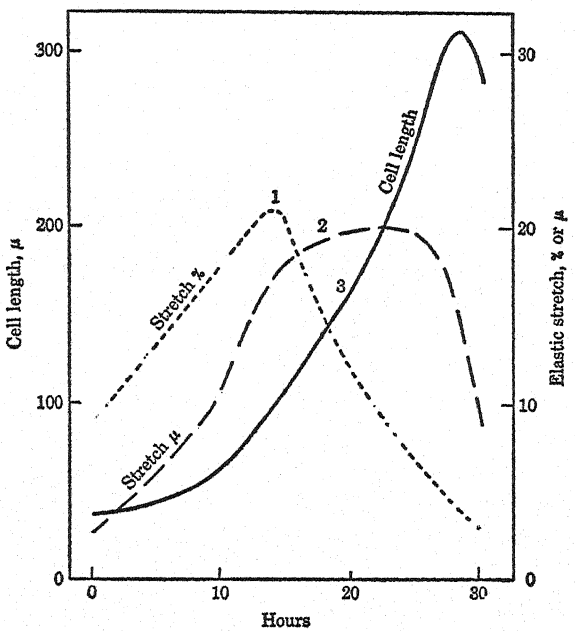


Fig. 39. Change in elastic stretch of root epidermis during cell elongation. Between 10 and 25 hours, the ability to stretch elastically is at a maximum (curve 2). During this same period, the growth rate (curve 3) is most rapid.

factor that must be considered: the resistance to the force. The mature cells have more rigid cell walls, the growing cells stretch more readily, and during cell growth the change in the rigidity is related to the rate of growth (Fig. 39). Consequently, a small turgor pressure is sufficient to stretch the walls of young, growing cells. But in order for cells to continue growth, other changes must also take place. In the simplest cases of cell growth there is nothing more than an uptake of water, producing a turgor pressure sufficient to overcome the force of attraction between the particles in the cell wall. As a result, the wall stretches and becomes thinner, and the osmotic potential drops due to uptake of water which dilutes the cell contents. The consequent reduction in osmotic equivalent soon brings water uptake (and therefore growth) to a stop. This simplest type of growth can be induced by artificial application of certain growth substances. Yet under normal conditions, cells are able to increase in size more than 15-fold (Fig. 39). In these cases, as water enters the cell during enlargement (accounting for about 90 per cent of the increase in cell size), solutes are also absorbed. In this way the cell maintains a high enough osmotic potential and a sufficient turgor pressure to stretch its wall (Table 24).

Besides water and solute absorption, other changes occur during cell growth. New wall material must be laid down by *intussusception*, an insertion of new particles between the old wall particles that have become separated. Cell thickening by *apposition* (a deposit of new particles on the inner surface of

TABLE 24: GROWTH AND SOLUTE CONTENT OF EPIDERMAL CELLS OF HELIANTHUS ANNUUS. EXPLANATION OF ZONES AS IN TABLE 23 [After Beck]

Zone	*Distance from cotyledons (mm.)*	*Average cell length (μ)*	*Average equivalent concentration (moles sucrose)*	*Total solutes per cell (arbitrary units)*
1	2.5	25.6	0.270	7.18
2	7.5	96.1	0.248	23.90
3	12.5	144.3	0.243	34.60
4	17.5	173.2	0.228	39.60
5	22.5	203.6	0.225	44.90
6	27.5	212.8	0.226	48.00
7	32.5	221.4	0.226	50.00

the wall) occurs during cell maturation (after enlargement has ceased).

The meristematic cell is filled with cytoplasm and has few small vacuoles. As enlargement progresses, the vacuole occupies a larger fraction of the cell, and the cytoplasm becomes a thin and almost invisible layer around it. This might lead one to suspect that no new cytoplasm is formed during cell growth. Such is not the case. In spite of the smaller fraction of the cell occupied by the cytoplasm, the latter increases as much as 4-fold in absolute amount (Blank and Frey-Wyssling).

Thus, though the simplest type of growth involves nothing more than a stretching of the wall due to an uptake of water, nevertheless under normal conditions the following processes all take place:

1. Absorption of water.
2. Absorption of solutes, maintaining the osmotic potential and turgor pressure practically constant.
3. Deposit of new wall material within the wall (intussusception).
4. Formation of new protoplasm (synthesis of proteins, phospholipids, etc.).

Growth is therefore a complex process, involving all or nearly all the other physiological processes occurring in the plant. It requires large quantities of energy, since absorption of solutes is probably active and therefore dependent on respiratory energy; and since the syntheses of cell wall and protoplasmic substances are endergonic processes.

Though the above classical concept of cell growth is logical enough and is based on considerable sound evidence, recent studies indicate that it is not always a true picture of the order of events. That cell growth can occur under artificial conditions according to the classical concept is easily proved. When potato slices are aerated in water their turgor pressure becomes so great that the cell walls are stretched beyond their elastic limits and a permanent increase in cell volume of as much as 50 per cent occurs. But this is not a normally growing tissue. Roots grow with such a low turgor pressure (about 1.5 atm. according to Burström) that the cell wall is not even stretched elastically,

let alone plastically. The root cells therefore grow in spite of the fact that the turgor pressure is too small to produce growth. Instead of being the initiating force in growth, turgor is here only a secondary factor. The initial cause of growth must be insertion of new material within the cell wall (intussusception), leading to increase in the perimeter of the cell. The turgor pressure would then serve simply to keep the wall taut, to prevent it from wrinkling as its perimeter increases. This would still be an essential role, for in the absence of turgor pressure, the wall particles might soon become too close together to permit any further intussusception.

In the case of many types of cells (e.g., development of vascular cells from cambial cells), growth appears to be uniform. In others (e.g., elongation of epidermal cells) growth seems to be localized at the tips (Frey-Wyssling). The protoplasm penetrates the loose cellulose framework in their tips, and even moves ahead of it, laying down a new framework as the cell elongates. The new cellulose framework is in the form of long strands that comprise the weft (parallel to the elongating cell). When the extending tip pushes further ahead, the warp (the cross strands) is laid down in the wall, reinforcing it. All this represents only the formation of the primary wall. The secondary wall is later formed by apposition.

Stages of growth

The growth of a plant or plant part characteristically passes through stages represented by an S-shaped curve (Fig. 40). The time during which this occurs has been called by Sachs the *grand period of growth.* Several attempts have been made to develop a mathematical expression for the growth curve. Blackman assumed that the shape of the curve results from the fact that the rate of production of new materials is proportional to the size of the plant. It has long been recognized that when growth results from cell division (and enlargement of the daughter cells to the size of the mother cell), each cell is replaced by two cells of the same size as the mother cell, and this process is repeated, so that there is a geometric progression

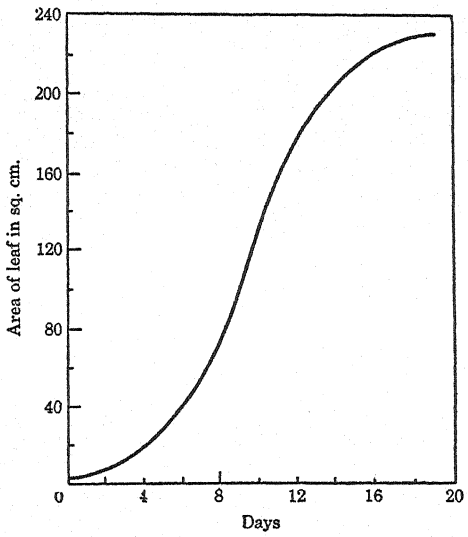


Fig. 40. Growth (in area) of cucumber leaf. (After Gregory)

(2, 4, 8, 16, 32, etc.). Blackman applied the compound interest formula to explain growth:

$$A = ae^{rt}$$

or in logarithmic form

$$\log_e \frac{A}{a} = rt, \quad \text{or} \quad 2.3026 \log \frac{A}{a} = rt$$

where A = final size
a = initial size
e = base of natural logarithms
r = rate of interest (or growth)
t = time interval

Thus, if a plant has doubled itself in 10 days, $A/a = 2$, and since $\log_e 2 = 0.69315$, the rate of increase, or growth, was therefore

$$r = \frac{\log_e (A/a)}{t} = \frac{0.69315}{10} = 0.0693$$

or 6.93 per cent per day. If the period of doubling were 5 days, the rate would be 13.8 per cent per day. But this constant rate

of growth can obviously apply only to the straight-line portion of the growth curve.

Robertson assumed that growth is based on a chemical transformation of assimilated or reserve substances into living protoplasm. As soon as it is formed, the new protoplasm begins to participate in these transformations, thus catalyzing its own synthesis. Consequently, he applied the formula for monomolecular, autocatalytic reactions, assuming that a single *master reaction* limits the growth rate. His equation for growth is

$$\frac{dx}{dt} = K(A - x) \quad \text{or} \quad \underline{\log} \frac{x}{A - x} = k(t - t_1)$$

where K = empirical constant
x = size reached in t days from beginning of growth
A = final size
t_1 = time to reach one-half final size

The curve obtained for this equation resembles the typical S-shaped growth curve. But only the simplest growth curves, or only portions of the more complex agree with Robertson's equation (Thompson).

Other formulas have been proposed by various workers, but all fail to express adequately the progress of growth, since they do not take into account all the processes and variables involved.

Influence of external factors on growth

Temperature. Growth of higher plants occurs in the range of about 0–35°C. Within most of this range, raising the temperature 10°C. increases the growth rate 2–3 times. There are three temperature zones known as the *cardinal points* for growth:

TABLE 25: CARDINAL POINTS (°C.) FOR GROWTH OF DIFFERENT PLANTS
[After Palladin]

Plant	*Minimum*	*Optimum*	*Maximum*
Barley	5	29	38
White mustard	0	21	28
Scarlet runner bean	10	34	46
Maize	10	34	46
Gourd, squash	14	34	46

the *minimum*, *optimum*, and *maximum*. These are not sharp temperatures and they vary from species to species (Table 25).

It should be pointed out that the optimum temperature for growth as determined in these tests is not necessarily the optimum for the general development and yield of the plant.

Light. Though the growth of higher plants eventually depends on photosynthesis, nevertheless light is not essential for the growth process itself, so long as sufficient quantities of organic substances are available. But the kind of growth is different when light is absent. In the dark, higher plants show a weak, spindly growth known as *etiolation*. In the case of most dicotyledons, the stem is excessively elongated, the leaves are underdeveloped. Little differentiation occurs, the tissue remaining mostly parenchymal. Usually the leaves remain free of chlorophyll, and the color is therefore pale yellow. Some nongreen plants show a similar stretching in darkness. Monocotyledons may show excessive elongation of the first internode and normal or excessive leaf development.

Relatively short, daily exposures to light prevent etiolation. Consequently, light must retard this excessive growth. An extreme case is the dwarfing of alpine plants by the intense light they are subjected to. This light is richer in violet and ultraviolet radiations, which appear to have more of a stunting effect than other wavelengths.

In spite of this commonly observed retardation of growth by light, the effect in many cases is just the opposite. Though the stems of etiolated plants are excessively long, the leaves may fail to grow altogether (e.g., potato sprouts). According to Thomson, the primary effect of light is to *accelerate* growth when cells are in the division stage. It is only after cell enlargement has progressed to near its normal limit that light retards any further excessive enlargement that would occur in the dark.

Water. Since all growth depends on a hydrostatic turgor pressure, a water deficiency will, of course, retard or completely stop it. On the other hand, an excess of water may result in an abnormal type of growth. Thus, in a saturated atmosphere the development of leaves is poor and the differentiation of the tissues is retarded. This is undoubtedly due to excessive stretch-

ing of the cell walls as a result of the abnormally high turgor pressure. Plants adapted to such conditions (e.g., aquatics) have low osmotic potentials and therefore cannot develop such excessive turgor pressures even when their tissues are saturated.

Chemical Stimulants and Inhibitors. Even the nutrient salts required by plants for normal growth may inhibit growth or actually kill the plants if applied in unbalanced solutions. On the other hand, they of course stimulate growth when applied in suitable quantities and in balanced solutions. Salts of the heavy metals (Cu, Pb, Ag, Hg, etc.) are particularly toxic. Even the metabolic products of a plant (e.g., oxalic acid) may be poisonous when supplied to the protoplasm instead of being stored in the vacuole. In very weak doses, many poisons actually stimulate growth. Thus phenol is poisonous in 1:1000 concentration, but stimulates when used in 4–8:100,000; ethyl alcohol checks growth in 25–75:1000, stimulates it in 25–75:100,000 (Maximov). Mercury compounds used to disinfect seeds sometimes stimulate growth. The subject of chemical stimulants and inhibitors will be returned to later when considering so-called *growth substances.*

Dormancy and rest period

Some of the above environmental factors may completely stop growth. This is most commonly the case in nature when the plant temperature is below the minimum for growth (e.g., in winter), or when its water content is too low (e.g., seeds). In this state of suspended growth, the plant is said to be *dormant.* But even when the environmental conditions are favorable for growth, the plant may still fail to grow because of some internal factors. In this state, it is said to be in a *rest period.* Twigs collected in fall, or newly harvested potato tubers fail to grow though the temperature, water, and other environmental factors are optimum for the growth of twigs and tubers not in their rest period. In some cases, part of the plant may grow while another part is arrested, for example, in the early growth of grasses, the leaves grow but the internodes do not. Similarly, lateral buds of trees may be dormant (more correctly "at rest") while the terminal bud is growing.

The rest period can be broken by artificial means. Thus lateral buds are *forced* by removing the terminal bud. Ether treatment (0.5 ml. per liter of air for 24–48 hr.) or warm baths (30–35°C for 9–12 hr.) are sometimes effective. These effects are believed due to the induction of anaerobic respiration and the consequent production of chemicals, such as acetaldehyde and ethyl alcohol, that may be able to break the rest period. But the effect is purely local; if one branch is treated, only this part of the plant will grow. Subjection to low temperatures, sometimes accompanied by alternate freezing and thawing, may also break the rest period, e.g., in the case of twigs and seeds. Thiocyanates and ethylene chlorhydrin are particularly effective in breaking the rest period of potato tubers. Light may stimulate the germination of some seeds (e.g., Poa, tobacco, carrot, Oenothera) and may retard others (e.g., Crataegus). In some cases, the rest period of seeds may simply be due to an impermeable seed coat. In these and others, excision of the embryos may overcome it.

Internal factors may lead to senescence in plants; the growth rate may then decrease and finally stop. This is obviously true in the case of annual and biennial plants that die at the end of their growth cycle even though external factors are still favorable for growth. It has even been maintained (Molisch) that vegetatively reproduced plants become senescent and gradually lose the vigor of their growth. Consequently, varieties of plants that are propagated in this way may eventually die out.

REFERENCES

1. Beck, W. A. Production of solutes in growing epidermal cells. Plant Physiol., **16**:637–642. 1941.
2. ——— and Basilia Andrus. The osmotic quantities of the cells in the hypocotyl of Helianthus annuus seedlings. Bul. Tor. Bot. Club, **70**:563–598. 1943.
3. Blackman, V. H. The compound interest law and plant growth. Ann. Bot., **33**:353–360. 1919.
4. Blank, F. and A. Frey-Wyssling. Protoplasmic growth in the hypanthium of *Oenothera acaulis* during cell elongation. Ann. Bot., **8**:71–78. 1944.

5. BURSTRÖM, HANS. Mechanism of cell elongation. In: Plant growth substances, ed. F. Skoog. University of Wisconsin Press. 1951.
6. BURSTRÖM, H. Studies on the growth and metabolism of roots. IX. Cell elongation and water absorption. Physiol. Plant., 6:262–275. 1953.
7. CROCKER, W. Growth of plants. Reinhold. 1948.
8. FREY-WYSSLING, A. Growth of plant cell walls. In: Structural aspects of cell physiology. Symp. Soc. Exp. Biol., 6:320–328. 1952.
9. GREGORY, F. G. The increase in area of leaves and leaf surface of Cucumis sativus. Ann. Bot., 35:93–123. 1921.
10. MAXIMOV, N. A. Plant physiology. McGraw-Hill. 1938.
11. MOLISCH, H. The longevity of plants. Edmund H. Fulling. 1938.
12. PALLADIN, V. I. Plant physiology, ed. B. E. Livingston. Blakiston. 1923.
13. ROBERTSON, T. B. The chemical basis of growth and senescence. Lippincott. 1923.
14. THOMPSON, D'ARCY W., On growth and form. Cambridge University Press, 1942.
15. THOMSON, BETTY F. The effect of light on the rate of development of Avena seedlings. Am. J. Bot., 37:284–291. 1950; 38:635–638. 1951.

Chapter 18

GROWTH MOVEMENTS

Though plants are usually thought of as stationary organisms, they are capable of many different kinds of movements. As in the case of stomatal movement, some, such as the rapid folding of the leaves of the sensitive plant when touched, and the slower but similar "sleep" movements of other legumes, are due to a more or less rapid loss of turgor. Other movements are due to differential rates of growth and are therefore much slower. Some of the most fundamental facts about growth have been discovered by the many investigations of these growth movements. It is for this reason that growth movements occupy such an important place in plant physiology.

There are three main types of growth movements: (1) *circumnutation*, (2) *tropisms*, (3) *nastic movements*. All or nearly all plants show circumnutation. This is a spiral type of growth of the plant apex, due to a different rate of growth on opposite sides of the growing tip. The more rapid rate of growth travels around the tip, which as it grows upward (in the case of the stem) must therefore rotate. The cause of this cyclic change in rates of growth of cells on different sides of the tip is not known.

In the case of the other two types of growth movements, the environmental factor responsible for the growth difference is definitely known and is spoken of as the *stimulus*. The length of time that the plant must be exposed to the stimulus is called the *presentation time*, the time for the reaction to occur is the *reaction time*, and the time it takes for the plant to recover its original position after the stimulus has been removed is the *relaxation time*.

In the case of tropisms, the direction of movement shows a definite relation toward, away from, or at a definite angle to the direction of application of the stimulus. A movement toward the stimulus is called a positive movement, away from

the stimulus, a negative movement. Nastic movements, on the other hand, show no definite directional relation to the stimulus.

Since movements can be caused by different stimuli, the name of the movement consists of a prefix which indicates the stimulus, and a suffix which states the kind of movement. The following tropic movements are known:

Movement	*Stimulus*
Geotropism	gravity
Phototropism	light
Chemotropism	chemicals
Hydrotropism	water
Thigmotropism Haptotropism	touch

Geotropism

Primary roots are *positively geotropic* (i.e., bend toward the center of the earth), primary stems are *negatively geotropic.* Secondary lateral roots and shoots show a weaker response and take up a position at an angle to the gravitational force. They are said to be *plagiogeotropic* as opposed to the primaries which are *orthogeotropic* (parallel to the gravitational force). Lateral roots and shoots of a higher order are practically insensitive to the stimulus of gravity (*apogeotropic*). Rhizomes grow horizontally, that is, they take up a position at right angles to the gravitational stimulus and are called *diageotropic.* The response of an organ to the gravitational stimulus may change with the stage of development. The peduncle of the poppy bud is positively geotropic but it gradually becomes negative as the flower opens. A change may also be induced artificially; if the primary root or stem tip is cut off, the lateral nearest to the wound becomes orthogeotropic.

The first sign of a geotropic response occurs a short distance back from the apex, except in grasses (and other monocots), in which case it occurs at the nodes where the meristems are found. If roots or shoots are *decapitated* (the apex is removed), they fail to show a geotropic response. If, however, they are exposed to the stimulus and then decapitated before curvature can occur (after the presentation time but before the reaction time) the stumps will nevertheless show a geotropic response.

Thus the tip is necessary for the *perception* of the stimulus but not for the *response*. This control of the responses of one part of a plant by another part is known as *correlation*.

Phototropism

As a rule, stems are positively phototropic, leaves are plagiophototropic. The majority of roots are insensitive. The peduncle of *Lynaria cymbalaria* is positively phototropic when the flower opens, negatively phototropic after fertilization. As a result, the capsule is pressed into cracks between rocks, where the seed may later germinate.

Etiolated coleoptiles of grasses (e.g., oats) have been most used for experimental work on phototropism. As in the case of geotropism, it is easily shown that the region of perception and the region of response to the light stimulus are not the same. The sensitive zone is the first 1 to 1.5 mm. from the tip.

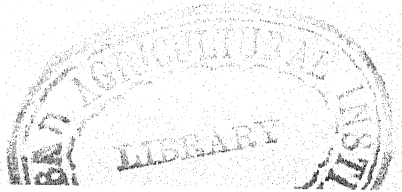
Phototropism is in accord with what has been said above about the retarding effect of light on cell elongation. The darkened side grows more rapidly and as a result curvature is toward the light. This, however, is a superficial and not uniformly correct explanation.

Other tropisms are similar to the above two but have not been studied so thoroughly.

Nastic movements

The commonest of these are *nyctinastic* movements, the day and night movements of leaves and flowers. Flower perianths and many compound leaves may open during the day and close at night. If these are growth movements, they are, of course, possible only while the organ is capable of growth. The amplitude of movement, therefore, decreases with age. But some retain the ability to open and close even when mature. This is because some nastic movements depend on turgor changes rather than growth differences. Families typically showing nyctinastic leaf movements are: Leguminosae, Oxalidaceae, Euphorbiaceae, Marantaceae.

Nyctinastic movements may be controlled by temperature, light, etc. The perianths of tulip, crocus, and other similar



flowers open at a constant temperature when illuminated and close when darkened. If, on the other hand, the light is kept constant, they open at high temperatures and close at low temperatures. They are therefore both *photonastic* and *thermonastic*. In each case, the opening is the result of a more rapid growth of the upper surface (*epinasty*). Such nastic movements may also be caused by the presence of certain substances in small quantity. One of the most sensitive tests for ethylene is the epinasty of tomato petioles, which occurs if the ethylene is present in 1:500,000 parts of air.

REFERENCES

1. THOMAS, M. Plant physiology. J. & A. Churchill. 1949.
2. WEEVERS, TH. Fifty years of plant physiology. Chronica Botanica. 1949.

Chapter 19

GROWTH SUBSTANCES

Both geotropic and phototropic reactions are characterized by one region of perception and another of response. This phenomenon is known as correlation. It has been conclusively shown by various methods that correlation is due to the transfer of a chemical substance from the region of perception to the region of response (Fig. 41). When the coleoptile tips of oat seedlings are exposed to light, a substance is released in the tip and is translocated downward to the region of response. One method of showing this is to cut off the tip after the presentation time, place it on agar for a while, then transfer the agar to the decapitated coleoptile. Curvature always occurs toward the side of the agar that was under the illuminated side of the tip.

The substances responsible for this correlation are known

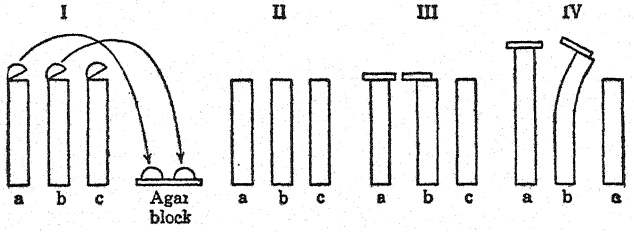


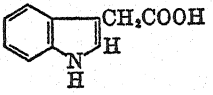
Fig. 41. Effects of decapitation and replacement of substances from tip (in agar blocks) on growth of oat coleoptiles. I, three coleoptiles decapitated; II, tips from *a* and *b* transferred to agar blocks; III, one block placed symmetrically on *a*, one asymmetrically on *b*, none on *c*; IV, *a* shows straight growth, *b* growth curvature, *c* no growth.

variously as *growth substances*, *hormones*, *auxins*, etc. The last of these three terms was coined to apply to all substances capable of inducing a curvature in the oat coleoptile. The term hormone is applied to any substance active in very low concentrations, that is synthesized in one part of an organism and

exerts its effect in another part. Since all kinds of effects may be involved, there may be many kinds of hormones other than those controlling growth.

Three substances have been isolated from plant material (and also from animal material) that are capable of producing this curvature. They are *auxin a*, *auxin b* (auxin a minus a molecule of water), and *heteroauxin*, or *indoleacetic acid*.

heteroauxin (indole-acetic acid)



When applied uniformly to decapitated coleoptiles, these substances cause increased growth. When applied to one side, they cause curvature toward the other side.

Because of the small amount of auxin in oat coleoptiles, it is difficult to prove conclusively which of the above three substances is responsible for normal growth. Some workers believe it is auxin a or b, others that it is indoleacetic acid, still others that it is a combination of both (Larsen). The most recent results point to indoleacetic acid as the main natural auxin (Bonner and Bandurski). In view of the uncertainty, it is perhaps safest tentatively to refer to the substance(s) simply as auxin.

As mentioned above, phototropic curvature is due to a higher concentration of auxin on the side of the coleoptile further from the light. This may be due either to translocation from the illuminated side or to photochemical destruction on this side. Geotropism can be similarly explained. The gravitational stimulus causes a greater concentration of auxin on the lower side. But it must be remembered that this causes negative geotropism in stems, positive in roots. This has been explained by the difference in sensitivity of roots and stems to the concentration of auxin (Fig. 42). The same concentration (10^{-6} molal) causes increased growth of stem cells, decreased growth of root cells. All cells may be either stimulated or inhibited, depending on the concentration of auxin. Recently, even nastic growth movements have been shown to be controlled by auxins (Brauner and Arslan).

This dual effect of auxin gives the plant a switch mechanism for turning the growth process on or off. The terminal bud is able to prevent the growth of laterals by liberating excess auxin to them. In this way buds may be kept in the dormant state. Resumption of growth by dormant buds or other organs may

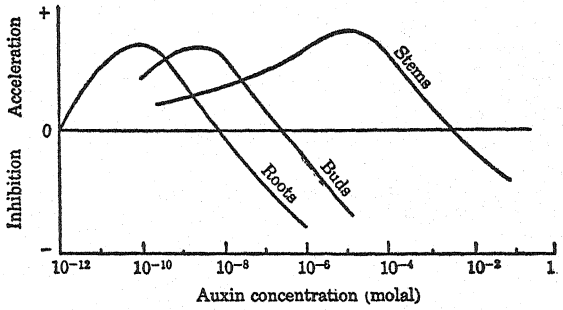


Fig. 42. Inhibition and acceleration of growth of different organs as a function of auxin concentration. (After Thimann)

result from the formation of an *antiauxin* in sufficient quantity to counteract part of the auxin and bring its effective concentration into the region of growth stimulation. The same result may be brought about by an auxin-destroying enzyme, *indoleacetic oxidase*. On the other hand, if the auxin is present in growth-stimulating quantity, the organ may be kept dormant by the presence of an antiauxin, for example, in the potato tuber (Hemberg) or in seeds (Mayer). The antiauxin in many seeds is a substance known as coumarin.

Mechanism of auxin action

In all the cases mentioned above, the effects of auxins on growth result from an increased or decreased cell enlargement at low or high concentration, respectively. It has long been known, however, that auxins may also affect cell division. Callus tissue may be formed when indoleacetic acid is applied to a cut stem or petiole surface. Similarly, root and fruit initiation (Fig. 43) is favored by the same substance, and fruit abscission can be prevented or induced. Renewed activity of cambium and apical meristems in spring seems to be dependent

on auxins. Any complete explanation of auxin action must include the effects on cell division and cell enlargement. Yet most theories have attempted primarily to explain the effect on cell enlargement.

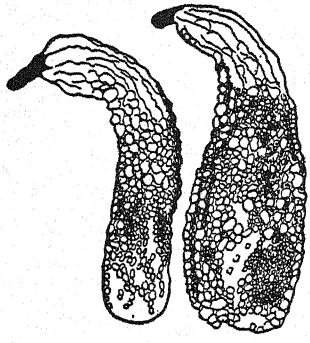


Fig. 43. Fruit formation in squash by treatment with 2% idolebutyric acid in lanolin (left) as compared with normal fruit by pollination (right). (After Gustafson; see Skoog)

This is perhaps understandable, since auxins apparently control the growth of only those organisms that show marked cell enlargement: the higher plants. They have no known effect on the growth of bacteria or fungi. Neither do they induce any growth response in organisms without cellulose cell walls, as for example, animals. These facts favor the view that auxins alter the cellulose cell walls in such a way as to lead to cell enlargement.

As mentioned above, cell enlargement can occur either if the force exerted on the cell wall by the turgor pressure is greater than the force of attraction between the particles of the cell wall, or if particles are inserted into the wall by intussusception. In either case, a cell may begin to enlarge as the result of either increased turgor pressure or decreased rigidity of the wall, or both. It has been amply proved that auxins do not cause any increased turgor pressure, but they do increase the *plasticity* of the wall. This, then, is one reason for the increased cell enlargement. In those cells that enlarge primarily as the result of intussusception, auxins must in some way be involved in the process. In both cases it is an effect on the wall.

Such an explanation, however, is by no means complete. The mode of action of the auxin on the cell wall is not at all clear. It has, however, been shown that auxins markedly affect respiration rate. Those concentrations of auxin that increase growth usually cause increased respiration, those that decrease growth usually cause decreased respiration, though there are exceptions, and it now appears that not the total respiration, but

only one part of it is of importance as far as auxin is concerned. Just how this effect on respiration is linked to the effects on the cell wall is impossible, as yet, to say. Perhaps the auxin effect on respiration releases the energy needed to break the bonds between the micelles of the cell wall, thus both increasing wall plasticity and permitting the intussusception of new wall material.

It should be pointed out that the auxin may be present in the plant in different forms. Indoleacetaldehyde may occur, and it is active in the same way as indoleacetic acid. But the auxin may also be bound in an inactive form, e.g., as a *protein-auxin complex.* Furthermore, one of the amino acids, tryptophane, is a precursor of indoleacetic acid and may therefore be converted to the latter in the plant.

Other growth substances have been found in the plant besides the auxins; for example, *traumatin* is the wound hormone that forms at the cut surface of potatoes. Still other substances of a hormone nature have been postulated to exist in the plant, as will be seen when the development of the plant is discussed. There are also *growth inhibitors* (e.g., coumarin) that, unlike the auxins, are unable to stimulate growth at concentrations lower than those that cause inhibition.

Besides the natural growth substances of the auxin type, there are many synthetic substances capable of producing the same effects on plant growth. By definition, they are also auxins, though not naturally occurring. Examples of these are indolebutyric acid, naphthaleneacetic acid, and the new important weed killer 2,4-D(2,4-dichlorophenoxyacetic acid). Quantitatively, some of these substances are more effective than the naturally occurring indoleacetic acid, some are less effective, depending on the test that is used; for there are many effects of both the natural and the synthetic substances. The substance that is most effective for one, may be least effective for another of these plant responses (Avery *et al.*).

Naturally occurring substances other than auxins may markedly affect the growth of plants though present in extremely small quantities (Fig. 44). They may therefore also be called growth substances, though the effects they produce are differ-

ent from the effects of the auxins. It is possible, for instance, to cut small pieces of roots from a plant and to grow these *excised* roots indefinitely in a suitable culture medium. This medium must, of course, contain all the essential mineral elements, as well as a sugar (since the roots do not photosynthesize). But, in most cases, it must also contain the substance thiamine (vita-

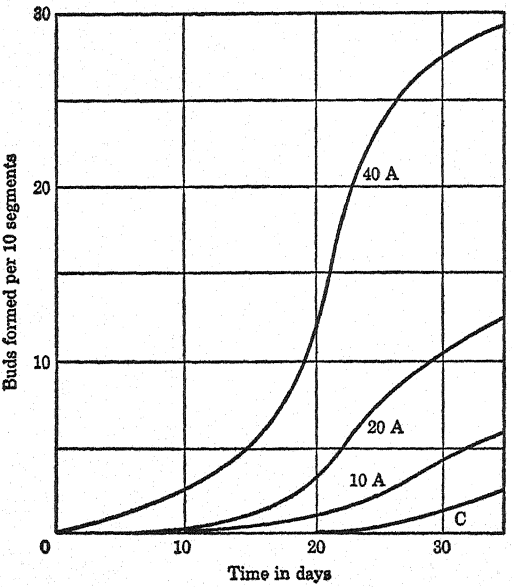


Fig. 44. Effect of adenine sulfate (40, 20, 10, and 0 milligrams per liter) on bud formation in tobacco stem segments. (After Skoog and Tsui; see Skoog)

min B_1) in quantities of about 0.1 mg. per liter. In the case of some species, the roots also require the vitamins pyridoxine (vitamin B_6) and nicotinic acid. There are other species (primarily monocots) whose roots have not as yet shown unlimited growth in such cultures, presumably because there are still other unknown growth factors that are missing. Similarly, tissues can be cut from apical or cambial meristems and grown in suitable culture media, forming so-called *callus tissue*. In this case, it may be necessary to add a mixture of substances of unknown composition, such as coconut milk, in order to obtain

unlimited growth (van Overbeek). So there must be some essential growth substances that have not as yet been discovered.

REFERENCES

1. AVERY, G. S., E. B. JOHNSON, R. M. ADDOMS, and B. F. THOMSON. Hormones and horticulture. McGraw-Hill. 1947.
2. BONNER, J. Plant biochemistry. Academic. 1950.
3. BONNER, J. and R. S. BANDURSKI. Studies of the physiology, pharmacology, and biochemistry of the auxins. Ann. Rev. Plant Physiol., **3**:59–86. 1952.
4. BOYSEN-JENSEN, P. Growth hormones in plants. McGraw-Hill. 1936.
5. BRAUNER, L. and N. ARSLAN. Experiments on the auxin reactions of the pulvinus of *Phaseolus multiflorus*. Revue de la faculté des sciences de l'université d'Istanbul Série B Tome XVI Fasc. **3**:257–300. 1951.
6. HEMBERG, T. The significance of the acid growth-inhibiting substances for the rest period of the potato tuber. Physiol. Plant., **5**:115–129. 1952.
7. HEYN, A. N. J. The physiology of cell elongation. Bot. Rev., **6**:515–574. 1940.
8. LARSEN, P. Formation, occurrence and inactivation of growth substances. Ann. Rev. Plant Physiol., **2**:169–198. 1951.
9. SKOOG, F. (ED.). Plant growth substances. University of Wisconsin Press. 1951.
10. THIMANN, K. V. On the nature of inhibitions caused by auxin. Am. J. Bot., **24**:407–412. 1937.
11. VAN OVERBEEK, J. Hormonal control of embryo and seedling. Cold Spring Harbor Symposia on Quant. Biol., **10**:126–134. 1942.
12. WENT, F. W. and K. V. THIMANN. Phytohormones. Macmillan. 1937.

Chapter 20

DEVELOPMENT

The callus tissue that is grown in artificial culture media may continue growth indefinitely without forming any specialized, differentiated tissues. It is thus capable of growth without development. The normal plant, however, undergoes both growth and development. After division and enlargement, the cells differentiate. The fertilized egg cell develops into an embryo, the seed develops into a seedling, the seedling develops into a flowering and then a fruiting plant. This may be followed by senescence and death.

It is only in relatively recent times that some of the physiological factors controlling the development of the plant have been discovered. And it is only the development from the vegetative to the reproductive stage that has received much attention.

In the past, several explanations of flowering have been proposed. At one time temperature was believed to be the sole factor. Later, the C:N ratio in the plant was suggested. But we now know that several factors operate.

Photoperiodism

In 1920 Garner and Allard showed that the flowering of many plants could be induced or prevented simply by controlling the length of the daylight period. Some plants flowered most rapidly when the day length was about 12 hours or less (*short-day* plants), others when the day length was about 12 hours or more (*long-day* plants), still others flowered at either day length (*day-neutral* plants). All types may occur in any one species, frequently producing so-called early (long-day) and late (short-day) varieties (Table 26). If the day length is unsuited to flowering, the plants may remain vegetative indefinitely, or they

may simply take much longer to flower (e.g., Peking and Biloxi, Table 26).

The photoperiodic effect has been found in all kinds of plants, herbaceous and woody, annual, biennial, and perennial, and recently even in animals. Besides reproduction, it affects many other phases of the physiology of the plant (Fig. 45). Trees do not enter their rest period in the fall if long days are maintained. As a result, they fail to develop *frost resistance* and are readily *winter-killed.* Biennials remain in the rosette form if subjected to short days. Vegetative as well as sexual reproduction is af-

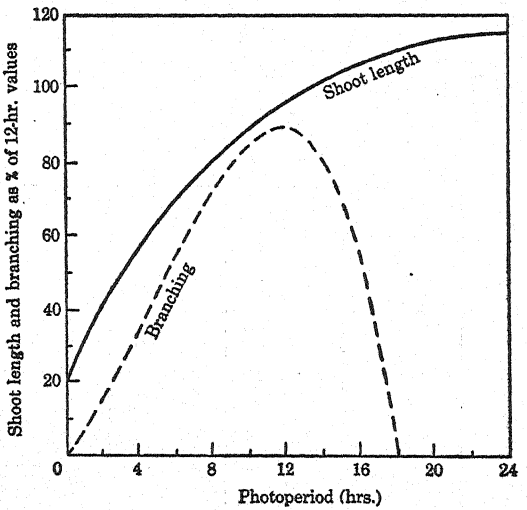


Fig. 45. Effect of photoperiod on vegetative development (shoot growth and branching) in the fiber plant Crotalaria juncea. (After Singh and Choudri; see Whyte)

fected. But the optimum for flowering may be different from the optimum for vegetative reproduction. Tuberization of potatoes occurs at shorter day lengths than maximum flowering.

It must be realized that the photoperiodic effect cannot substitute for photosynthesis. Consequently, the optimum day length for flowering will not be the optimum for yield in the case of short-day plants. In order to obtain maximum yield, they must first be grown under a long day to enable the accu-

TABLE 26: FLOWERING TIMES FOR SOYBEAN VARIETIES GROWN UNDER DIFFERENT DAYLENGTHS [Adapted from Garner and Allard]

Day length (*hr.*)	*Time (days) from germination to blossoming*		
	Mandarin (*day neutral*)	*Peking* (*short-day*)	*Biloxi* (*short-day*)
5	23	23	27
7	21	21	26
12	21	21	28
Full daylight (12½–15 hr.)	26	62	110

mulation of reserves and enlargement of the plant. Similarly, the temperature factor cannot be ignored (Fig. 46).

In the case of a long-day plant, flowering can be induced in natural short days by lengthening the daylight period with weak light. This subsidiary light is far too weak to induce photosynthesis. Similarly, long-day plants may be forced to

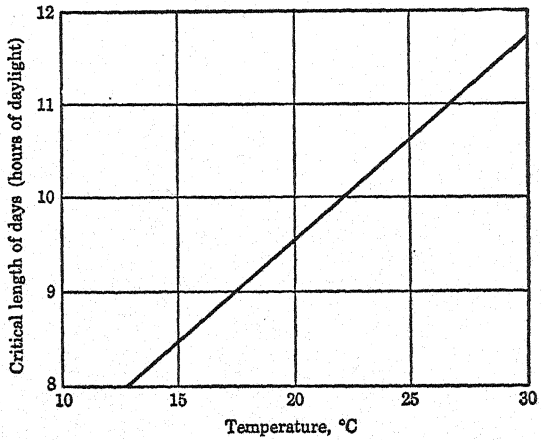


Fig. 46. Dependence of critical daylength for flowering on temperature in Hyoscyamus niger. (After Lang and Melchers)

flower in short days by interrupting the dark period with a few minutes of light. This and other evidence has proved that it is the dark period that inhibits flowering of long-day plants and stimulates flowering of short-day plants. Grafting experiments have been particularly effective in proving this (Lang).

Short-day plants will flower when exposed to long days, pro-

vided that they have already been exposed to a sufficient number of short days. Long-day plants will similarly flower during short days, if previously exposed to a sufficient number of long days. This carryover is called the *photoperiodic after effect* (or *photoperiodic induction*).

As in the case of growth itself there is strong evidence of a hormone control of development (Lang). The response occurs in the growing points, yet these do not have to be exposed to any particular day length. The region of perception is the leaf, and even a single one, if subjected to the necessary day length, may induce the growing points to flower. The floral stimulus may even be transferred from one plant to another by grafting. It is, therefore, generated in the leaf and translocated to the growing points. The light is apparently absorbed by a green pigment (distinct from chlorophyll) and the energy utilized either to synthesize a substance or to destroy one synthesized in the dark. Some believe the substance is the same one that controls growth (indoleacetic acid) and that it inhibits flowering. Others conclude that a specific substance (*florigen*) is involved. It is interesting to note that flowering has been controlled recently by several workers with the use of specific substances applied to specific plants (Lang).

In spite of the striking effects of the photoperiod, it is not the only factor that controls flowering. Temperature may also be a deciding factor.

Thermoperiodism

Tomatoes are day-neutral plants under ordinary conditions. But their flowering can be controlled by the day and night temperature. A night temperature of 15°C and a day temperature of 25°C are optimum for flowering. If these are changed too markedly from the optimum, flowering is reduced or even prevented. Other plants have shown similar responses. Went has called this phenomenon *thermoperiodism*.

Vernalization

Some winter annuals must be sown in fall in order to flower the following summer. If spring sown, they either fail to flower

the first year, or flower much later than when fall sown. If, however, the seed are moistened (with enough water to increase their weight about 60 per cent) and kept at 0°C–5°C for about a month, they can then be spring sown and will flower at about the same time as the fall sown seed. This treatment is known as *vernalization*. After vernalization, they still must be subjected to a suitable photoperiod (usually a long day) in order to flower.

Some biennials show a similar behavior toward temperature. If the young seedlings are exposed to low temperatures (about 5°C–10°C in the case of several species), they will subsequently flower the first year at a higher temperature, provided that the latter temperature is not too high. The actual temperature requirements vary considerably from species to species.

The mechanism of the temperature effect has not been studied so thoroughly as in the case of photoperiodism. Yet the evidence again indicates a hormonal control, and the name *vernalin* has been coined for the unknown substance. Lang suggests that vernalin is either a precursor of florigen or a catalyzer of its formation.

REFERENCES

1. Garner, W. W. and H. Allard. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. J. Agr. Research, **18**:533–606. 1920.
2. Lang, A. and G. Melchers. Die photoperiodische Reaktion von Hyoscyamus niger. Planta, **33**:653–702. 1943.
3. Lang, A. Physiology of flowering. Ann. Rev. Plant Physiol., **3**:265–306. 1952.
4. Murneek, A. E. and R. O. Whyte. Vernalization and photoperiodism. Chronica Botanica, 1948.
5. Whyte, R. O. Crop production and environment. Faber and Faber. London. 1946.

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